AFRL-RH-WP-SR-2009-0001

Proceedings of the 2006 Toxicology and Risk Assessment Conference: Applying Mode of Action in Risk Assessment

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July 2006

Interim Report for July 2005 to July 2006

Air Force Research Laboratory
711th Human Performance Wing
Human Effectiveness Directorate
Biosciences and Protection Division
Applied Biotechnology Branch
Wright-Patterson AFB OH 45433

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
July 2006	Interim	July 2005 – July 2006

4. TITLE AND SUBTITLE
Proceedings of the 2006 Toxicology and Risk Assessment Conference: Applying Mode of Action in Risk Assessment

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
62202F

6. AUTHOR(S)
*David R. Mattie
**Patricia A. Daunt
***Gary L. Foureman

5d. PROJECT NUMBER
2312

5e. TASK NUMBER
A2

5f. WORK UNIT NUMBER
2312A215

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
**U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, 26 W. Martin Luther King Drive, Cincinnati OH 45224
***U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, Hazardous Pollutant Assessment Group, B-243-01, Research Triangle Park NC 27711

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Air Force Materiel Command *
Air Force Research Laboratory
711th Human Performance Wing
Human Effectiveness Directorate
Biosciences and Protection Division
Applied Biotechnology Branch
Wright-Patterson AFB OH 45433-5707

10. SPONSOR/MONITOR'S ACRONYM(S)
711 HPW/RHPB

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
AFRL-RH-WP-SR-2009-0001

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES
88 ABW/PA cleared 7 February 2008, WPAFB-08-0265.

14. ABSTRACT
The 2006 Toxicology and Risk Assessment Conference was held April 25 and 26 at the Marriott North, Cincinnati, OH. The conference was co-sponsored by the Air Force (AFRL/Applied Biotechnology Branch, AFRL/Air Force Office of Scientific Research, and AFIOH/Health Risk Assessment Branch); Navy (NHRC/Environmental Health Effects Laboratory); Army (Center for Health Promotion and Preventive Medicine); U.S. Environmental Protection Agency, National Center for Environmental Assessment; Agencyfor Toxic Substances and Disease Registry/Division of Toxicology; and National Institute for Occupational Safety and Health-Cincinnati OH, along with the cooperation of the National Research Council/National Academy of Sciences. The theme for the conference was: "Applying Mode of Action to Risk Assessment," with the U.S. Environmental Protection Agency Assistant Administrator and Science Advisor, George M. Gray, PhD, delivering the key note address. The conference drew over 200 scientists throughout government, academia and industry. The conference has annually proven to provide networking opportunities and insight to toxicology.

15. SUBJECT TERMS
Toxicology, risk assessment, mode of action

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			David R. Mattie
U	U	U	SAR	87	19b. TELEPHONE NUMBER *(include area code)* NA

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. 239.18

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PREFACE

This technical report represents the proceedings of the 2006 Toxicology and Risk Assessment Conference with the primary sponsors being the U.S. Air Force, Air Force Research Laboratory, Applied Biotechnology Branch (AFRL/RHPB) and the U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment. Pat Daunt served as the conference coordinator for the U.S. Environmental Protection Agency.

The effort was conducted under Department of the Air Force Contract No F33615-00-C-6060. Dr. David R. Mattie served as the Contract Technical Monitor for AFRL/RHPB and Dr. Darol Dodd served as Program Manager for the ManTech/GEO-CENTERS Joint Venture Contract (F33615-00-C-6060). ManTech Environmental Technology, Inc. was acquired by Alion Science and Technology Corporation during this contract. Ms Tara Grove served as the conference coordinator for the Joint Venture Contract. As the contract ended prior to the conference, additional support was provided by Henry Jackson Foundation (HJF) under Air Force Contract No. FA8650-05-2-6518. Mark Hoffman served as the Contract Technical Monitor for AFRL/RHP and Thomas Scofield as the Program Manager for HJF. Ms Natalie Fielman was the conference coordinator for HJF. We would also like to acknowledge MaryAnn Angell, Paul Bloomer and John Rosenquist, all from HJF, for their support of the conference.

INTRODUCTION

The abstracts published in this technical report represent the basis and background of the presentations given at the 40th Annual Conference sponsored by Tri-Service Toxicology [Air Force (AFRL/Applied Biotechnology Branch, AFRL/Air Force Office of Scientific Research, and AFIOH/Health Risk Assessment Branch), Navy (NHRC/Environmental Health Effects Laboratory), and Army (Center for Health Promotion and Preventive Medicine)]; U.S. Environmental Protection Agency, National Center for Environmental Assessment; Agency for Toxic Substances and Disease Registry/Division of Toxicology; and National Institute for Occupational Safety and Health-Cincinnati, OH; along with the cooperation of the National Research Council/National Academy of Sciences. The 2006 Toxicology and Risk Assessment Conference was held April 25 and 26 at the Marriott North, Cincinnati, OH. The conference drew over 200 scientists throughout government, academia and industry.

The conference was opened with introductory remarks from the co-chairs, several of which alluded to the impact of this meeting on the scientific toxicological community. One co-chair (GF) analogized this meeting to a habitat interface occurring instead as a discipline interface between toxicology and risk assessment. As with all such interfaces, this also has resulted in especially rich, varied and fruitful outcomes due to the infusion of such broad intellectual diversity. With the anticipation of new insights, ideas, concepts and friendships that could develop in this habitat, the conference was declared open.

The keynote address during the opening plenary session on Applying Mode of Action to Risk Assessment was delivered by George M. Gray, Ph.D., Assistant Administrator and Science Advisor for the U.S. Environmental Protection Agency. Dr. Gray's presentation centered on the challenges involved in conducting risk assessments, the importance of understanding mode of action and uncertainty, and the need for research and data that address the most relevant uncertainties. Dr. Gray emphasized that this conference had, over the course of its existence, become a substantive part of the solution to understanding toxicological risk and was part of the way forward.

There were seven continuing education workshops over a range of risk assessment topics including: Chemical Mixtures; Epidemiologic Fundamentals; Replacing Default Values for Uncertainty Factors with Chemical Specific Adjustment Factors; Reducing Uncertainty in Noncancer Risk Assessment; Guidelines for Ecological Risk Assessment; Benchmark Dose Methods; Fundamentals of Risk Assessment; and Multi-Criteria Decision Analysis Tools for Managing Complex Environmental Challenges. In addition there were two discussion sessions: "Chemicals and Substances of Common and Emerging Concern"; and "You Say Tomato; I Say Lampshade - Different Methods and Mechanisms for Health Risk Assessment." The program, which is included in this report, gives an overview of each workshop and discussion session.

The program also gives an overview of each of the nine sessions followed by the presentations in that session: Plenary Session on Mode of Action; Hierarchical Linkages in Ecological Assessment; Internal Dose; Genome Based Risk Assessment Adversity in Sensitive Populations; Heavy Metals; Mode of Action in Cancer Risk Assessment; Hearing Loss from Noise and Chemicals; Mode of Action in Metals - Arsenic and Organotin Compounds; Toxicology, Health Effects, Hazard Identification of Nanoparticles; and Chemical Mixtures.

There was also a Poster Session on topics related to the conference. Combined with the poster session was a student competition for best poster. Conference co-chairs were Gary Foureman, Ph.D., US EPA/ORD/NCEA and David Mattie, Ph.D., AFRL/RHPB.

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PROGRAM

2006 Toxicology and Risk Assessment Conference

Applying Mode of Action in Risk Assessment

April 24 - 27, 2006

Conference Co-Chairs: Mattie, David R., Ph.D., D.A.B.T., *Air Force Research Laboratory, Applied Biotechnology Branch*

Foureman, Gary L., Ph.D., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*

Monday, April 24, 2006 **1:00 p.m. - 5:00 p.m.**

Workshops

Workshop Chair:
Yu, Kyung O., Ph.D., *Air Force Research Laboratory, Applied Biotechnology Branch*

11:30 a.m. - 5:00 p.m. **Registration**

1:00 p.m. - 5:00 p.m. **Workshops W-1, W-2, W-3 and W-4, Discussion Session**

2:30 p.m. - 3:00 p.m. **Break**

W-1. Intermediate Topics on Health Risk Assessment of Chemical Mixtures
Presenters:
Teuschler, Linda K., M.S., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*
Hertzberg, Richard C., Ph.D., *Tetra Tech EM, Inc.*
Rice, Glenn E., M.S., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*
Mumtaz, Moiz M., Ph.D., D.A.B.T., *Agency for Toxic Substances and Disease Registry*

This half-day workshop presents intermediate topics and hands-on exercises on risk based methodologies for assessing cumulative health risk from exposure to chemical mixtures, including considerations of multiple route exposures and toxicological interactions. Included are limited descriptions of basic concepts, the introduction of cutting edge chemical mixture health risk assessment risk issues, explanation of state-of-the-art methods, and hands on exercises for several important classes of chemical mixtures (e.g., pesticides, metals, drinking water disinfection by-products). Workshop topics include: procedures and definitions for selecting among risk assessment methods; methods for incorporating toxicologic interactions data (weight of evidence for interactions; the interaction-based hazard index); discussions of

exposure issues unique to chemical mixtures (e.g., environmental transformations); use of physiologically-based pharmacokinetic modeling of interactions and multiple route exposure assessment; and integrating relative potency factors with response addition for mixtures of chemicals representing similar and dissimilar toxic modes of action. The content of this workshop includes a general overview of chemical mixture health risk assessment data evaluation and procedures, a detailed description of several new methods, and in-depth hands-on exercises with test data sets. Discussions include real world examples, exercise results, issues for application of the procedures, and general questions and comments. Participants are asked to bring a calculator.

W-2. Epidemiologic Fundamentals for Risk Assessment Applications

Presenters:
Murphy, Patricia A., Ph.D., M.P.H., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*
Wright, J. Michael, Sc.D., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*

Data and information from epidemiologic studies can be used qualitatively and quantitatively in the different phases of risk analysis. This is a half-day workshop devoted to the introduction of epidemiologic principles and general applications of epidemiological information in risk analysis. The workshop is targeted to the non-epidemiologist working in the general area of environmental health risk assessment. The goal for workshop participants is to become intelligent consumers of epidemiologic information and recognize opportunities for its appropriate application in different risk assessment activities.

Participants will be introduced to elements of epidemiologic study design and the interpretation and calculation of common measures of association. The impact of bias and confounding on relative risk estimates will also be examined. Discussions will focus on identifying strengths and limitations of different types of human data. The last part of the workshop will focus on a practical exercise in assessing study validity and drawing causal inferences from observational epidemiologic data.

W-3. Replacing Default Values for Uncertainty Factors with Chemical Specific Adjustment Factors: Reducing Uncertainty in Noncancer Risk Assessment

Presenters:
Lipscomb, John C., Ph.D., D.A.B.T., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*
Haber, Lynne, Ph.D., D.A.B.T., *Toxicology Excellence for Risk Assessment*

The World Health Organization, through the International Programme on Chemical Safety (IPCS), has recently established guidance on the use of mechanistic data to replace default uncertainty factors for interspecies extrapolation and intraspecies variability in deriving risk values such as Reference Doses (RfDs) and Tolerable Concentrations (TCs). This guidance informs the choice and application of data that can be used to replace defaults with chemical specific adjustment factors (CSAFs). CSAFs fall on the continuum of the use of data in deriving risk values. At one end of the continuum is the use of the traditional defaults, while at the other end is the use of extensive chemical-specific data in physiologically based pharmacokinetic (PBPK) modeling or even biologically-based dose-response (BBDR) modeling. In between these two extremes is the use of categorical defaults, such as the dosimetric approach used in

the U.S. EPA's RfC and cancer risk assessment methods and CSAFs. The CSAF framework is based on early work by Renwick and is applied by IPCS. This approach first subdivides the uncertainty factors for interspecies differences (UFA) and human variability (UFH) into toxicokinetic (TK) and toxicodynamic (TD) components. The data relevant for each subcomponent is then evaluated to determine whether chemical-specific data can be used in place of the default. Any one or all of these four subfactors can be replaced by chemical-specific data. In the absence of chemical-specific data, default values of 2.5 and 4.0 have been established for the TD and TK component of UFA, while the default values for the TD and TK components of UFH were each established at one-half order of magnitude (3.2). Use of the CSAF framework allows the improved use of available data in deriving risk values, and can assist in targeting new studies to address uncertainties and lead to more accurate risk values. CSAFs have been used by the U.S. EPA in deriving an RfD for boron and by Health Canada in deriving a TC for 2-butoxyethanol. This half-day workshop will provide a brief review of the use of uncertainty factors and historical perspective on the reliance on quantitative data to develop values for inter- and intraspecies extrapolation. The course will focus on the IPCS methodology for CSAF development, including the thinking process and steps used for evaluating data. Examples and classroom activities will be used as instructional aids.

W-4. Guidelines for Ecological Risk Assessment

Presenter:
Sergeant, Anne, M.E.S., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*

This half-day Guidelines for Ecological Risk Assessment seminar is intended for those wanting an overview of the ecological risk assessment process. It describes the basics of ecological risk assessment with emphasis on planning to ensure that the results are useful in risk-management decisions, and allows time for discussion and a straightforward case exercise or two. The workshop starts with a brief history of ecological risk assessment and comparison with human-health risk assessment, and then explores the processes of planning, problem formulation, analysis, risk characterization, and how to relate risk-assessment results to risk-management decisions. It also includes several case examples.

Discussion Session: Chemicals and Substances of Common and Emerging Concern

Presenters:
Mattie, David R., Ph.D., D.A.B.T., *Air Force Research Laboratory, Applied Biotechnology Branch*
Johnson, Mark S., Ph.D., *U.S. Army Center for Health Promotion and Preventive Medicine, Health Effects Research Program*

National defense requires the use of substances that are often not common in general industrial use (e.g., energetics, propellants, explosives, etc.). Training and testing of equipment that uses these substances have resulted in the contamination of the environment at varying levels in various media. Since these substances are military unique, data necessary for a complete evaluation of health risks, both ecological and human, are often not complete. Participants of this discussion group will discuss chemical compounds of common concern that require additional data to help reduce uncertainty and to accurately assess the risks from exposure, both human and ecological. Known exposure consequences, identification of remaining research needs for establishing reasonable exposure standards and regulatory issues will be

addressed. Updates on issues, relevant organizations and status of highest priority chemicals will be presented or made available as well.

6:00 p.m. – 10:00 p.m. **Social Event – Reds Hall of Fame and Dinner at the Riverfront Club**

Tuesday, April 25, 2006 **8:00 a.m. – 11:45 a.m.**

Morning Session

8:00 a.m. – 8:30 a.m. **Opening Remarks**

- Preuss, Peter, Ph.D., *Director, National Center for Environmental Assessment*, *Office of Research and Development, U.S. EPA*
- Schlager, John J., Ph.D., D.A.B.T., *Air Force Research Laboratory, Applied Biotechnology Branch*
- Kozumbo, Walter J., Ph.D., *Air Force Research Laboratory, Air Force Office of Scientific Research*
- Long, G. Cornell, M.S., *Air Force Institute of Operational Health, Health Risk Assessment Branch*
- Chapman, Gail D., CDR, MBA, Ph.D., *Medical Service Corps, U.S. Navy, Naval Health Research Center Detachment, Environmental Health Effects Laboratory*
- Roszell, Laurie E., Ph.D., D.A.B.T., *U.S. Army Center for Health Promotion and Preventive Medicine*
- Woebkenberg, Mary Lynn, Ph.D., *National Institute for Occupational Safety and Health, Division of Applied Research and Technology*
- Fowler, Bruce A., Ph.D., Fellow A.T.S., *Agency for Toxic Substances and Disease Registry, Senior Biomedical Research Service*
- Bakshi, Kulbir, Ph.D., *National Academy of Sciences/National Research Council's Committee on Toxicology*

8:35 a.m. – 11:45 a.m. **Plenary Session**

9:45 a.m. – 10:15 a.m. **Break**

1. Approaches and Advances in Application of Mode of Action Information to Risk Assessment Processes

Co-Chairs:
Foureman, Gary L., Ph.D., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*
Mattie, David R., Ph.D., D.A.B.T., *Air Force Research Laboratory, Applied Biotechnology Branch*

This session is devoted to highlighting the commitment and recent advances that the scientific and regulatory community has made towards installing mode of action as the fundamental basis for decisions made in the risk assessment process. The keynote address given by Dr. Gray

highlighted the utility and prominence of mode of action at the EPA as a scientific basis for integrating toxicological information and in addressing uncertainty about this information. He also noted that both of these aspects are becoming more and more vital in the decision making processes in the EPA. The presentations following explore fundamentals and application of mode of action in risk assessment. These include characterization of the relationship of dose to mode(s) of action, a presentation on endpoint characterization, leukemia, approached from the aspect of utilizing mode of action principles, as well as a presentation demonstrating the utility of mode of action approach for a specific agent, methyl iodide.

8:35 a.m. **Mode of Action and Uncertainty Analysis as Emerging Issues in Risk Assessment at the U.S. EPA**
Gray, George M., Ph.D., *Office of Research and Development, U.S. EPA, Assistant Administrator and Science Advisor*

9:15 a.m. **Effect Transitions in Dose Response/Low Dose Extrapolation**
Slikker, William, Jr., Ph.D., *Food and Drug Administration*

9:45 a.m. **Break**

10:15 a.m. **Induced Leukemias: Agents, Subtypes, and Insights into Carcinogenic Modes of Action**
Eastmond, David A., Ph.D., *University of California, Environmental Toxicology and Department of Cell Biology*

10:45 a.m. **Application of Mode of Action Data in a Risk Assessment for Methyl Iodide**
Gargas, Michael L., Ph.D., *The Sapphire Group™*

11:15 a.m. **Panel Discussion**

11:45 a.m. – 1:00 p.m. **Lunch**

Tuesday, April 25, 2006 **1:00 p.m. – 5:00 p.m.**

Afternoon Sessions

1:00 p.m. – 5:00 p.m. **Sessions 2A, 2B and 2C**

3:00 p.m. – 3:30 p.m. **Break**

2A. **Winding Thread into Rope: How Can We Fortify Hierarchical Linkages in Ecological Assessment?**
Co-Chairs:
Shaw-Allen, Patricia L., Ph.D., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*
Reed, David A., Ph.D., *U.S. Army Center for Health Promotion and Preventive Medicine*

An organism's response to stressors is not always well-linked with ecologically relevant effects. This is one of the pervasive conflicts in ecological assessment. Responses to stressors are

only correlatively linked with the changes in vital rates that drive population and higher order effects. A good mechanistic understanding of metabolism and mode of action allows defensible cause/effect correlations between toxicant exposure and biochemical responses. However, extrapolation to higher order effects beyond biochemical responses can be suspect. Determining the importance of non-chemical stressors such as altered resource availability and habitat quality is even more complex.

We need approaches that integrate complex stressor responses that may result in ecological consequences as well as approaches that disaggregate ecological information if we are to diagnose the principal causes of ecological problems.

In this session, scientists working at various scales of the ecological hierarchy describe their approaches and share their perspectives on this issue.

1:00 p.m. **Introduction**
Shaw-Allen, Patricia L., Ph.D., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*

1:15 p.m. **From Molecules to Populations: Using Population Genetics to Answer the So What Question**
Bagley, Mark, Ph.D., *U.S. EPA, Office of Research and Development, National Exposure Research Laboratory*

1:50 p.m. **Linking Biochemical Responses to Ecological Consequences: Can Nitrogen Isotope Ratios in Tissues Do the Job?**
Shaw-Allen, Patricia L., Ph.D., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*

2:25 p.m. **Towards a Completely New and Radically Different ERA Paradigm**
Tannenbaum, Larry, M.A., *U.S. Army Center for Health Promotion and Preventive Medicine, Environmental Health Risk Assessment Program*

3:00 p.m. **Break**

3:30 p.m. **Using Quantile Regression to Develop Stressor-Response Relationships for Community Metrics and Bedded Sediments from Field Data**
Griffith, Michael B., Ph.D., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*

4:05 p.m. **Multiple Land Use Effects on Upland Forest Vegetation of a Heterogeneous Military Site**
Collins, Beverly S., Ph.D., *University of Georgia, Savannah River Ecology Laboratory*

4:40 p.m. **Panel Discussion**

2B. Internal Dose: The Ultimate but Elusive Determinant of Risk

Co-Chairs:
Gearhart, Jeffery M., Ph.D., D.A.B.T., *The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., Air Force Research Laboratory, Applied Biotechnology Branch*
Snawder, John E., Ph.D., *National Institute for Occupational Safety and Health, Biological Monitoring Laboratory Section*

This meeting emphasizes the new horizons on toxicology that are being expanded by application of mode of action (MOA) information. As exemplified in the plenary session, however, the dose-response character of the MOA events is complementary and necessary for a full evaluation of MOA information. Further, the more specific the description of the dose, the more complete the evaluation. The NRC, in its landmark 1994 publication, "Science and Judgment in Risk Assessment," clearly and unequivocally identifies for the risk assessment community that in humans " ... *the target-site dose is the ultimate determinant of risk*..."

This session demonstrates the span of various methods and approaches now being applied to obtain the actual dose to the target site or to approximate a relevant internal dose as a surrogate for the dose to the target site. Discussions include a novel example of the validity of the target tissue - dose response relationship, use of adducted products in blood as surrogates for target-site dose, the use of physiological and anatomical constructs to estimate dose to target tissues (respiratory tract) from inhalation exposures, the applicability of cross-species allometry for internal dosimetry as well as an in depth prospective on the use and limitations of physiologically based pharmacokinetic (PBPK) models in determining the ultimate determinant of risk.

1:00 p.m. **Introduction**
Snawder, John E., Ph.D., *National Institute for Occupational Safety and Health, Biological Monitoring Laboratory Section*

1:05 p.m. **Internal Dose and Response in Real-Time**
Boyes, William K., Ph.D., *U.S. EPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory*

1:45 p.m. **Approaches to Determining Internal Dose in Inhalation Risk Assessment at the U.S. EPA**
Stanek, John, Ph.D., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*

2:25 p.m. **Internal Dose Selection: Identification of Risk-Relevant Dose Metrics and the Impact on Extrapolation**
Sweeney, Lisa M., Ph.D., D.A.B.T., *The Sapphire Group™*

3:00 p.m. **Break**

3:30 p.m. **Assessment of Internal Dose from Hemoglobin Adducts**
Fennell, Timothy R., Ph.D., *Research Triangle Institute*

4:05 p.m. **Prospects and Limitations for the Use of Mode of Action Information and Biological Modeling to Characterize Internal Dose**
Clewell, Harvey J., III, Ph.D., *CIIT Centers for Health Research*

4:40 p.m. **Panel Discussion**
Rappoteur: Clewell, Harvey J., III, Ph.D., *CIIT Centers for Health Research*

2C. Genome Based Risk Assessment Adversity in Sensitive Populations

Co-Chairs:
Fowler, Bruce A., Ph.D., Fellow A.T.S., *Agency for Toxic Substances and Disease Registry*
Savage, Russell E., Jr., Ph.D., *National Institute for Occupational Safety and Health*

There is growing scientific interest in the application of modern "omic"–based biomarkers for risk assessments following exposure to individual chemicals and chemical mixtures. These technologies are capable of facilitating identification of populations at special risk for toxicity/cancer based upon genetic polymorphisms in specific pathways, genetic susceptibility for diseases and gender differences in genetic responses to chemical exposures. In order to be of maximal value for risk assessment purposes, it is important for putative biomarkers to be validated across species and for early molecular alterations to be ultimately linked to the onset of clinical disease. Speakers in this session will examine these issues from a variety of molecular endpoints and link these changes to clinical outcomes and ultimately to risk assessment calculations. Such calculations or analysis will provide useful information on the degree to which molecular biomarkers may be predictive of health outcomes on a population basis.

1:00 p.m. **Introduction**
Fowler, Bruce A., Ph.D., Fellow A.T.S., *Agency for Toxic Substances and Disease Registry*

1:15 p.m. **A Mechanistic Approach to Identify Polymorphic Genes in Pathways Associated with Risk of Brain Cancer**
Butler, Mary Ann, Ph.D., *National Institute for Occupational Safety and Health*

1:50 p.m. **Genetic Susceptibility Risk Factors for Polygenic Diseases**
Demchuk, Eugene, Ph.D., *Agency for Toxic Substances and Disease Registry*

2:25 p.m. **The Role of Genetic Variations in Occupational Lung Diseases**
Yucesoy, Berran, Ph.D., *National Institute for Occupational Safety and Health*

3:00 p.m. **Break**

3:30 p.m. **Gender Differences in Proteomic Responses to III-V Semiconductor Particles**
Fowler, Bruce A., Ph.D., Fellow A.T.S., *Agency for Toxic Substances and Disease Registry*

4:05 p.m. **Risk Analyses for Benzene Using Molecular Biomarker Data**
Hack, C. Eric, M.S., *Toxicology Excellence for Risk Assessment*

4:40 p.m. **Panel Discussion**

Tuesday, April 25, 2006 **5:30 p.m. – 7:30 p.m.**

Evening Session

Poster Session/Reception
Poster Session Co-Chairs:
Westrick, Mark P., Major, M.S., CLS, CLDir, *U.S. Air Force, Air Force Research Laboratory, Applied Biotechnology Branch*
Daunt, Patricia A., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*

Wednesday, April 26, 2006 **8:00 a.m. – 11:45 a.m.**

Morning Session

8:00 a.m. – 11:45 a.m. **Sessions 3A, 3B and 3C**

9:45 a.m. – 10:15 a.m. **Break**

3A. Heavy Metals of Emerging Toxicological Concern
Co-chairs
Roszell, Laurie E., Ph.D., D.A.B.T., *U.S. Army Center for Health Promotion and Preventive Medicine*
Wilfong, Erin, LT, Ph.D., *U.S. Navy, Naval Health Research Center, Environmental Health Effects Laboratory*

Due to environmental and human health issues, heavy metals including lead and uranium are being phased out of certain military applications and replaced with "safer," "greener" materials. This includes certain military munitions, which are now manufactured from tungsten and tungsten alloys. Historically, tungsten was assumed to pose no health or environmental risks. However, there is growing concern that tungsten and tungsten alloys, which are composed of tungsten and nickel, cobalt, iron, copper and/or tin alloying agents, may not be as benign as previously thought. Presentations in this session will concentrate on recent toxicology studies involving tungsten, tungsten alloys, nickel, cobalt and other heavy metals commonly used in military applications.

8:00 a.m. Introduction
Roszell, Laurie E., Ph.D., D.A.B.T., *U.S. Army Center for Health Promotion and Preventive Medicine*
Wilfong, Erin, LT, Ph.D., *U.S. Navy, Naval Health Research Center, Environmental Health Effects Laboratory*

8:15 a.m. Carcinogenic and Toxicological Effects of Embedded Tungsten and Tungsten Alloys/Overview of Heavy Metals of Interest to the Army
Roszell, Laurie E., Ph.D., D.A.B.T., *U.S. Army Center for Health Promotion and Preventive Medicine*

8:45 a.m. **Industrial Hygiene Aerosol Sampling**
Isenstein, Mark D., M.H.S., *U.S. Army Center for Health Promotion and Preventive Medicine*

9:15 a.m. **The Acute Effects of Tungsten Alloys (WA) on the Airway**
Wilfong, Erin, LT, Ph.D., *U.S. Navy, Naval Health Research Center, Environmental Health Effects Laboratory*

9:45 a.m. **Break**

10:15 a.m. **Induction of Morphological Transformation and Global Disruption of Gene Expression in C3h/10t1/2 Mouse Embryo Cells by Specific Insoluble Nickel Compounds**
Landolph, Joseph R., Jr., Ph.D., *University of Southern California, Keck School of Medicine, Cancer Research Laboratory*

10:45 a.m. **Molecular Biomarkers for Evaluating Metal/Metalloid Interactions: An Overview**
Fowler, Bruce A., Ph.D., Fellow A.T.S., *Agency for Toxic Substances and Disease Registry*

11:15 a.m. **Toxicogenomics As a Tool for Identifying Biomarkers and Assessing Mechanisms of Action of Toxic Metals**
Hamilton, Joshua W., Ph.D., *Dartmouth Medical School*

3B. **Issues and Application of Mode of Action in Cancer Risk Assessment**
Co-Chairs:
Keshava, Nagu, Ph.D., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*
Toraason, Mark, Ph.D., *National Institute for Occupational Safety and Health*

Use of mode of action (MOA) information in the assessment of potential carcinogens is a major focus of the EPA's recently published Guidelines for Cancer Risk Assessment and Supplemental Guidance for Early Life Exposures. Due to significant scientific advances concerning the causes of cancer induction, elucidation of an MOA for a particular cancer response in animals or humans can inform human health risk assessments in a number of ways. For instance, MOA information helps to inform the extrapolation of laboratory animal-based toxicity results to humans. Understanding the MOA can also be a key to identifying processes that may cause chemical exposures to differentially affect a particular population segment or life-stage. However, the increasing availability of the MOA data raises several issues as to its application to risk assessment. The following presentations will illustrate a number of these issues through a discussion of the available MOA information and using examples of case studies. Particular issues include: (1) characterizing the MOA for a particular response to a specific chemical exposure; (2) human relevance analysis of information on carcinogenic MOA; and (3) determining whether an MOA supports particular approaches to low dose extrapolation. Furthermore, for all these issues, a key judgment in the analysis and interpretation of the data on MOA is an evaluation of when there is sufficient information to make risk assessment decisions based on one or another MOA hypothesis.

8:00 a.m. **Introduction: Use of Mode of Action in Risk Assessment - Brief Overview of EPA's 2005 Cancer Guidelines**
Keshava, Nagu, Ph.D., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*

8:15 a.m. **The Use of Carcinogenic Mode of Action Data in Human Health Risk Assessment**
Flowers, Lynn, Ph.D., D.A.B.T., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*

8:45 a.m. **A Framework for Human Relevance Analysis of Information on Carcinogenic Modes of Action**
Meek, M.E. (Bette), Ph.D., *Health Canada, Safe Environments Programme*

9:15 a.m. **Use of Genetic Toxicology Data in Establishing a Carcinogenic Mode of Action**
Schoeny, Rita, Ph.D., *U.S. EPA, Office of Water*

9:45 a.m. **Break**

10:15 a.m. **Application of Mode of Action Data in Risk Assessment – Inorganic Arsenic**
Ramasamy, Santhini, Ph.D., D.A.B.T., *U.S. EPA, Office of Water*

10:45 a.m. **Recent Insights into Benzene's Mode of Action and Their Implications for Cancer Risk Assessment and Low Dose Extrapolation**
Eastmond, David A., Ph.D., *University of California, Environmental Toxicology and Department of Cell Biology*

11:15 a.m. **Probing the Mode of Action of Tumorigenic Conazoles Using Traditional and Toxicogenomic Approaches**
Nesnow, Stephen, Ph.D., *U.S. EPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory*

3C. **Assessing the Risk of Hearing Loss from Noise and Chemicals**
Co-Chairs:
Morata, Thais C., Ph.D., *National Institute for Occupational Safety and Health*
Teuschler, Linda K., M.S., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*

While noise is the predominant source of work-related hearing loss, recent evidence has demonstrated that chemical toxicants can also cause hearing loss and enhance sensitivity to noise. This physical-chemical interaction has been documented enough to be reflected in recommendations put forth by NIOSH, ACGIH and the U.S. Army. It has also impacted legislation, as in February 2003 the European Parliament published a new noise directive (2003/10/EC), to be adopted by all participants' countries by 2006. This Directive requires employers, when carrying out risk assessment, to give attention to any effects on workers' health and safety resulting from interactions between noise and work-related ototoxic substances.

This session is directed to toxicologists, industrial hygienists, hearing loss prevention researchers and professionals from labor, industry and government. It will be a forum for the current knowledge of chemical ototoxicity to be reviewed by experts in the area. Time will be given to allow participant discussions on how to address chemical exposures in novel hearing prevention efforts required by the new directive.

8:00 a.m. **Introduction**
Teuschler, Linda K., M.S., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*

8:15 a.m. **The Solvent Effects on the Auditory Efferent Pathway**
Campo, Pierre, Ph.D., *Institut National de Recherche et de Sécurité, France*

8:45 a.m. **Ototoxic Stress: Styrene-induced Hearing Loss**
Chen, Guang-Di, Ph.D., *SUNY, Center for Hearing and Deafness*

9:15 a.m. **Multi-disciplinary Techniques in the Assessment of Neurotoxic Effects of Solvents on the Central Auditory System**
Gopal, Kamakshi V., Ph.D., *University of North Texas, Department of Speech and Hearing Sciences*

9:45 a.m. **Break**

10:15 a.m. **Chemical Exposure as a Risk Factor for Hearing Loss: Implications for Occupational Health**
Morata, Thais C., Ph.D., *National Institute for Occupational Safety and Health*

10:45 a.m. **U.S. Army CHPPM Guidelines for Preventing Hearing Loss from Chemical Exposures**
Carroll, Christopher H., M.S.E.S., C.I.H., *U.S. Army Center for Health Promotion and Preventive Medicine*

11:15 a.m. **Panel Discussion**

Luncheon Session

11:45 a.m. – 1:00 p.m. **Lunch**

An Overview of Federal, State and Local Public Health Investigations of Trichloroethylene Contamination Sites
Moffett, Daphne B., CDR, Ph.D., *U.S. Public Health Service, Agency for Toxic Substances and Disease Registry*

Afternoon Sessions

1:00 p.m. – 5:00 p.m. **Sessions 4A, 4B and 4C**

3:00 p.m. – 3:30 p.m. **Break**

4A. Mode of Action in Metals in Risk Assessment: Confounders and Implications of Arsenic and Organotin Compounds

Co-Chairs:
Mudipalli, Anuradha, Ph.D., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*
Preston, R. Julian, Ph.D. , *U.S. EPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory*
Lipscomb, John C., Ph.D., D.A.B.T., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*

Drinking water remains an important exposure route for many contaminants, including metals and organometallic compounds. Arsenic (As) and four organotin compounds will be covered in this session, which will emphasize the development and application of understandings of modes of action, exposures, and dose response evaluations. The first half of the session will focus on As, with talks devoted to characterizing several modes of action, including those involving signal transduction, transcription factors, gene regulation of proliferative factors, oxidative stress, and inhibition of DNA repair; the role of confounding environmental and genetic factors, with an emphasis on environmental physical stressor such as UV and an elucidation of research needs and strategies for integrating potential interactions among multiple and concurrent modes of action and confounders in the context of risk assessment. The second half of the session will be devoted to an evaluation of four organotin compounds (mono- and d- substituted methyl and butyl tins). These compounds are stabilizers of PVC pipe used in residential plumbing systems for drinking water; they are simultaneously encountered and their risk assessment will be performed as a mixtures-based approach. The initial talk will present and discuss data and modeling approaches to characterize human exposures to these compounds via drinking water; the second talk will present and summarize toxicity findings from each of the four compounds. The final presentation will provide an overview of approaches to chemical mixture risk assessment, and use those approaches as a framework to integrate toxicological findings from the four compounds to enable a chemical mixtures risk assessment for these four organotin compounds.

1:00 p.m. **Introduction – Arsenic Cancer Risk Assessment**
Preston, R. Julian, Ph.D*., U.S. EPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory*

1:15 p.m. **Arsenic Carcinogenicity – Mode of Action**
Mudipalli, Anuradha, Ph.D., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*

1:50 p.m. **On The Mechanism of Arsenic-Associated Skin Cancer**
Rossman, Toby G., Ph.D., *Nelson Institute of Environmental Medicine and NYU Cancer Institute, New York University School of Medicine*

2:25 p.m. **Interpretation of Biomonitoring Studies to Assess Exposure and Risk of Inorganic Arsenic: Confounding by Other Sources of Arsenic**
Beck, Barbara D., Ph.D., D.A.B.T., *Gradient Corporation*

3:00 p.m. **Break**

3:30 p.m. **Modeling Human Exposure to Organotins in Tap Water via Migration from PVC and CPVC Piping**
Fristachi, Anthony, M.S., *Department of Energy, Oak Ridge Institute for Science and Education*

4:00 p.m. **Toxicology Of Mono- and Di-alkyltin Chlorides**
Murphy, Sandi R., Ph.D., *Arkema, Inc.*

4:30 p.m. **Application of Mode of Action and Dose-Response Information in a Mixtures Risk Assessment**
Lipscomb, John C., Ph.D., D.A.B.T., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*

4B. Nanoparticles: Toxicology, Health Effects, Hazard Identification
Co-Chairs:
Hussain, Saber M., Ph.D., *Air Force Research Laboratory, Applied Biotechnology Branch*
McCain, Wilfred C., Ph.D., *U.S. Army Center for Health Promotion and Preventive Medicine*
Simeonova, Petia P., M.D., Ph.D., *National Institute for Occupational Safety and Health*

Nanotechnology is the key technology of the modern century. The possibility to exploit the structures and processes of biomolecules for novel functional materials, biosensors, bioelectronics and medical applications has created the rapidly growing field of nanobiotechnology. Although there is a wide application of nanomaterials, there is a serious lack of information concerning the human health and environmental implications of manufactured nanomaterials. Occupational health risks associated with manufacturing and using nanomaterials are not yet clearly understood. The rapid growth of nanotechnology is leading to the development of new materials, devices and processes that lie far beyond our current understanding of environmental and human impact. Exposure to these materials during manufacturing and use may occur through inhalation, dermal contact and ingestion. There is a lack of information on exposure routes, potential exposure levels and material toxicity. There is need to bring toxicologists, cell biologists, physical scientists, and engineers together to discuss the following aspects in the emerging field of nanotechnology. The integrated approaches from multidisciplinary fields of nanotechnology will provide better understanding of future impact on human health. The main focus of this session would be to discuss the following aspect of nanotechnology with a special emphasis on occupational exposure and risk assessment of nanomaterials.

- Potential routes for human exposure and level of exposure
- Industrial sources of occupational exposure
- Environmental fate and transport of nanostructures
- Health and safety aspects of nanoparticles
- Interaction of nanostructures and biological systems
- Integration of emerging methodologies to assess nanomaterial health effects

- NanoToxiconformatics that integrates approaches from multidisciplinary fields of nanotechnology
- Development of Nanotoxicity Knowledge Data Base (NKDB).

1:00 p.m. **Introduction**
Hussain, Saber M., Ph.D., *Air Force Research Laboratory, Applied Biotechnology Branch*

1:15 p.m. **Risk Assessment and Medical Surveillance**
Schulte, Paul A., Ph.D., *National Institute for Occupational Safety and Health*

1:50 p.m. **Nanoparticles are Capable of Producing Reactive Oxygen Species, Causing Increased Cytotoxicity, and Altering Gene Expression**
Oberdörster, Günter, D.V.M., Ph.D., *University of Rochester, Department of Environmental Medicine*

2:25 p.m. **Challenges Associated with Health Risk Assessment of Nanomaterials: More Than Just Size**
Dreher, Kevin L., Ph.D., *U.S. EPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory*

3:00 p.m. **Break**

3:30 p.m. **Physical and Chemical Characterization of Nanoparticles**
McMurry, Peter H., Ph.D., *University of Minnesota, Department of Mechanical Engineering*

4:05 p.m. **Nanomaterial Exposure and Risk for Systemic Effects**
Simeonova, Petia P., M.D., Ph.D., *National Institute for Occupational Safety and Health*

4:40 p.m. **Panel Discussion**

4C. New Approaches for Assessing the Health Effects from Exposures to Chemical Mixtures

Co-Chairs:
Robinson, Peter J., Ph.D., *The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., Air Force Research Laboratory, Applied Biotechnology Branch*
Rice, Glenn E., M.S., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*

In this session, new approaches developed to estimate the risks associated with exposures to environmental chemical mixtures will be presented. Pollutants have traditionally been addressed individually rather than as they actually occur - as mixtures. The session focus will be both on methods used to estimate exposures to multiple chemicals in environmental media and on approaches to examine the interactions of these chemicals in the body, following absorption. This will include analyses of mechanisms of interactions. The session will consist of presentations on screening level assessments and advanced assessments of chemical mixtures of concern to government agencies and the general public.

1:00 p.m. **Introduction**
Robinson, Peter J., Ph.D., *The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., Air Force Research Laboratory, Applied Biotechnology Branch*

1:15 p.m. **Non-additive Interactions of an Organophosphorus Pesticide Mixture in Adult and Preweanling Rats**
Moser, Virginia C. (Ginger)., Ph.D., D.A.B.T., *U.S. EPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory*

1:50 p.m. **The Effects of Jet Fuel on the Airway and Immune Function**
Wilfong, Erin, LT, Ph.D., *U.S. Navy, Naval Health Research Center, Environmental Health Effects Laboratory*

2:25 p.m. **Low-Dose Mixture Effects of PCB126 and Perchlorate on the Male Rat Hypothalamic-Pituitary-Thyroid (HPT) Axis**
McLanahan, Eva D., B.S., *Graduate Student, University of Georgia*

3:00 p.m. **Break**

3:30 p.m. **Simulation Modeling of Pharmacological Intervention for Chemical Warfare Agent Exposure (CWA) Affects**
Gearhart, Jeffery M., Ph.D., D.A.B.T., *The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., Air Force Research Laboratory, Applied Biotechnology Branch*

4:00 p.m. **PBPK Model for Selected Aromates in a JP-8 Vapor Exposure System**
Campbell, Jerry L., Ph.D., *The University of Georgia*

4:30 p.m. **Physiologically Based Pharmacokinetic Modeling of the Neurotoxicity of Mixtures**
Clewell, Harvey J., III, Ph.D., *CIIT Centers for Health Research*

Thursday, April 27, 2006 **8:00 a.m. - 4:00 p.m.**

Workshops

Workshop Chair:
Yu, Kyung O., Ph.D., *Air Force Research Laboratory, Applied Biotechnology Branch*

8:00 a.m. - 4:00 p.m. **Workshop W-5, W-6, W-7**

8:00 a.m. - 12:00 p.m. **Discussion Session**

10:00 a.m. - 10:20 a.m. **Break**

2:10 p.m. - 2:30 p.m. **Break**

1 hour 15 minute **Lunch Break**

W-5. Introduction to Benchmark Dose Methods and Hands-on Application of EPA's Benchmark Dose Software (BMDS)

Presenters:
Gift, Jeffrey S., Ph.D., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*
Howard, Angela, Ph.D., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*
Zhao, Q. Jay, M.P.H., Ph.D., D.A.B.T., *Toxicology Excellence for Risk Assessment*

Part I - Introduction to Benchmark Dose Methods (morning)

Benchmark dose methods (BMD) are gaining considerable favor among the EPA and other risk assessors for, among other things, establishing a point-of-departure for use in quantifying the noncancer and cancer risk from chemical exposures. Attendees will receive an overview of the EPA's benchmark dose approach as is currently outlined in the 2000 draft of the Agency's Benchmark Dose Technical Guidance Document and as it applies to the recently finalized 2005 EPA cancer guidelines. The course will cover definitions of important terms and will provide an overview of the BMD process, including determination of data adequacy, model fitting, model comparison, selection of a benchmark response level and modeling linear versus nonlinear responses.

Part II - Hands-on Application of the EPA's Benchmark Dose Software (BMDS) (afternoon)

Attendees will receive information, via actual chemical assessment examples, concerning practical applications and pitfalls of the BMD approach and the use of the Agency's benchmark dose software (BMDS) version 1.4. Through this training course, participants will learn basic and more advanced techniques for model fitting, including making full use of model options, considering covariates, re-initializing model parameters to achieve maximum fit and restricting or specifying model parameters to achieve more plausible fits. This workshop will cover all the BMD models available in current version of the EPA's BMDS including continuous models, dichotomous models and nested models. Attendees will also receive tips on how to organize their BMDS files during more complex BMD chemical assessments.

W-6. Fundamentals of Risk Assessment

Presenters:
Reid, Jon, Ph.D., D.A.B.T., *U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment*
Lipscomb, John C., Ph.D., D.A.B.T., *U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment*
Rice, Glenn E., M.S., *U.S. EPA, Office of Research and Development, National Center for Environmental Assessment*
Fristachi, Anthony, M.S., *Department of Energy, Oak Ridge Institute for Science and Education*

This day-long course is intended for those with only basic knowledge of human health risk assessment methodology. The course has many class problems demonstrating the methodology; an emphasis will be placed on classroom discussions. The Risk Assessment paradigm will be presented and steps described. Lecture topics will include hazard identification; dose-response (toxicity) including a discussion of mode of action for cancer and noncancer effects; pharmacokinetic approaches to species extrapolation, exposure and risk characterization. Instruction will be aided by the presentation of data for a hypothetical chemical with both cancer and noncancer effects. Students will be given an oral and an inhalation

exposure scenario and an evaluation of risk will be undertaken as a class exercise. A detailed analysis of an environmentally relevant chemical will complete the workshop. Students will leave with practical knowledge of the EPA standard methodology for risk assessment. A calculator is required.

W-7. Multi-Criteria Decision Analysis Tools for Managing Complex Environmental Challenges

Presenters:
Linkov, Igor, Ph.D., *Cambridge Environmental Inc., Carnegie Mellon University*
Ferguson, Elizabeth, Ph.D., *U.S. Army Corps of Engineers, Engineer Research and Development Center*
Kim, Jongbum, Ph.D., *U.S. Army Corps of Engineers, Engineer Research and Development Center*
Suedel, Burton, Ph.D., *U.S. Army Corps of Engineers, Engineer Research and Development Center*
Kiker, Greg, Ph.D., *University of Florida, Agricultural and Biological Engineering Department*

Decision-making in environmental projects is typically a complex and confusing exercise, characterized by trade-offs between socio-political, environmental and economic impacts. Cost-benefit analyses (CBA) are often used, occasionally in concert with comparative risk assessment, to choose between competing alternatives. However, assessment of remedial, abatement and land-use policies for contaminated sites involves multiple criteria, such as cost, benefit, environmental impact, safety and risk, which may not be easily condensed into a single monetary value. Consequently, alternatives or trade-offs may be incomparable on a CBA basis. Even in cases where it is possible to convert multiple criteria into a common single unit, this approach would not always be desirable because competing or mutually exclusive stakeholder group preferences may be lost in the decision process. The overall effect is to foster a defensive decision-making culture among government agencies and private firms that may be slow to adopt or test new environmental technologies such as beneficial reuse of contaminated sediments or *in situ* treatments. As an alternative to CBA, multi criteria decision analysis (MCDA) and Comparative Risk Assessment (CRA) offer scientific and theoretically sound analytical decision methods. Recent workshops of federal stakeholders (U.S. Army Corps of Engineers, EPA, NOAA) have constructed basic frameworks that are applicable to environmental projects dealing with contaminated and disturbed sites where risk assessment and stakeholder participation are of crucial concern. This workshop will provide an interdisciplinary perspective of CRA and MCDA methods for the assessment of novel contaminated sediment management technologies in the context of public stakeholder values. Participants learn a basic overview of CRA and MCDA techniques and tools with some time allowed for work/consultation on specific issues of concern to participants.

Discussion Session. You Say Tomato; I Say Lampshade - Different Methods and Mechanisms for Health Risk Assessment

Presenter:
Reed, David A., Ph.D., *U.S. Army Center for Health Promotion and Preventive Medicine*

“Risk assessment” is being used and applied in an increasing array of professions and in increasingly different methods. It is, in fact, near to qualifying as a buzzword. Within the health risk community the term is solid; however, it also uses many different methods. This discussion

session will present and discuss the different methods and mechanics health risk assessors are using to generate health risk information within their respective areas of expertise. Planned topics will include ecological, combustion, homeland security and microbial risk assessment. As an interesting addition, a local risk assessor from outside the health field will also be asked to describe their work (details unknown). This could be a financial, corporate, energy or law enforcement assessor (suggestions welcome!). A great session for those new to the field looking for a broader base as well as experienced assessors seeking new ways for doing assessment!

SESSION 1: APPROACHES AND ADVANCES IN APPLICATION OF MODE OF ACTION INFORMATION TO RISK ASSESSMENT PROCESSES

Mode of Action and Uncertainty Analysis as Emerging Issues in Risk Assessment at the U.S. EPA

Gray, George M., Ph.D.,
U.S EPA, *Office of Research and Development -Assistant Administrator and Science Advisor*

Effect Transitions in Dose Response/Low Dose Extrapolation

Slikker, William, Jr.
Food and Drug Administration

Analysis of dose-response curves for many agents demonstrates that multiple mechanisms exist. For example, critical, limiting steps in any given mechanistic pathway may become overwhelmed with increasing exposures, signaling the emergence of new modalities of toxic tissue injury at higher doses. Kinetic- and/or dynamic-mediated responses may be altered in a non-linear manner with increasing dose. Chemical-specific case studies show that, as the dose of an agent increases, dose-dependent transitions such as receptor interactions, altered homeostasis, and saturation of pharmacokinetic and repair mechanisms can and do occur. Such dose-dependent transitions in the principal mechanism of toxicity could have significant impact on the interpretation of reference data sets for risk assessment. The existence of dose-dependent transitions calls into question the human relevance of effects seen at high doses in traditional toxicity testing, particularly if such doses do not reflect relevant human exposure levels.

Induced Leukemias: Agents, Subtypes, and Insights into Carcinogenic Modes of Action

Eastmond, David A.
University of California, Environmental Toxicology and Department of Cell Biology

Leukemia is one of the most common types of induced cancer and results from exposure to a range of chemical and physical agents including ionizing radiation, alkylating agents and topoisomerase II inhibitors. Rather than representing a single disease, leukemia represents a collection of diseases with unique characteristics. While the induced leukemias show certain similarities, they also exhibit a number of significant differences. The initial focus of the presentation will be an overview of the diverse classes of human leukemia-inducing agents with an emphasis on patterns of toxicity, types of leukemias induced in humans and animals, and the currently identified mechanisms underlying xenobiotic-induced leukemogenesis in humans. This framework will then be used to provide insights into the modes-of-action of other occupational and environmental agents such as formaldehyde, which have recently been implicated as causing leukemia.

Application of Mode of Action Data in a Risk Assessment for Methyl Iodide

Gargas, Michael L.[1]; Kinzell, John H.[2]
[1]The Sapphire Group™
[2]Arysta LifeScience North America Corporation

Recent studies have indicated that acute exposures to methyl iodide (MeI) produce a number of effects in laboratory animals, including fetal toxicity, neurotoxicity and degeneration of the nasal epithelium. An understanding of the mode of action (MOA) by which these effects are produced by MeI is necessary in guiding critical decisions used in the risk assessment. These include the selection of the appropriate internal dose measure(s) calculated using physiologically based pharmacokinetic (PBPK) modeling, and evaluating the relevance of the observations in animals to human health. Several potential MOAs were evaluated and compared for each endpoint using the modified Hill Criteria. These evaluations and comparisons indicated that: (1) fetal toxicity in rabbits is most likely a result of modulation of thyroid hormones due to elevated iodide concentrations in the fetus resulting from metabolism of MeI; (2) neurotoxicity observed in rats is most likely the result of a generalized inhibition of ion currents in the membranes of nerve cells due to high concentrations of parent chemical and (3) degeneration of the nasal epithelium of rats is dependent on the sustained depletion of GSH below critical levels due to the metabolism of MeI in the nasal tissues.

SESSION 2A: WINDING THREAD INTO ROPE: HOW CAN WE FORTIFY HIERARCHICAL LINKAGES IN ECOLOGICAL ASSESSMENT?

From Molecules to Populations: Using Population Genetics to Answer the So What Question

Bagley, Mark
U.S. EPA, Office of Research and Development, National Exposure Research Laboratory

Important endpoints for ecological risk assessments are usually those that affect population or species persistence rather than individual-level responses. Nonetheless, ecological risk assessments are generally based on measures of individual-level responses. Extrapolation of individual responses to populations via modeling approaches has received great interest but is limited by data and system understanding; environmental stressors are often multifactorial, with unknown interactive effects among variables, making it difficult to model population responses accurately. Empirical approaches to understanding environmental effects on demographic parameters and population dynamics are helpful but time-consuming and expensive. We have taken the approach of evaluating molecular population genetic patterns in conjunction with environmental variability data to understand population-level responses to changes in environmental condition. Genetic diversity patterns within and among populations result from integrated population responses to various environmental stressors as they impact effective population size, connectivity among populations, and local adaptation. Examples of the population genetic approach will be presented for stream fish populations assessed at the scales of watersheds and ecoregions. Ultimately, we expect our work to contribute to a framework for spatially explicit ecological risk assessment that integrates assessment of landscape stressors and habitat condition with genetic and population modeling approaches.

Linking Biochemical Responses to Ecological Consequences: Can Nitrogen Isotope Ratios in Tissues Do the Job?

Shaw-Allen, Patricia L.[1]; Romanek, Christopher S.[2]; Jagoe, Charles H.[3]
[1]U.S. EPA, Office of Research and Development, National Center for Environmental Assessment
[2]University of Georgia, Department of Geology
[3]University of Georgia, Savannah River Ecology Laboratory, Ecotoxicology, Remediation, and Risk Assessment Group

One of the pervasive conflicts in ecotoxicology and ecological risk assessment is the inability to rigorously link biochemical responses to toxicants with ecologically relevant effects, such as natality, growth and mortality rates. Biochemical responses are presumed to tax resources, such as protein, needed for growth and reproduction. Examining shifts in the stable isotopes of nitrogen (standardized $^{15}N/^{14}N$ ratio or $\delta^{15}N$) may address this problem. Due to the higher protein deamination and transamination rates of ^{14}N amines compared to their ^{15}N-bearing counterparts, protein decomposition increases $\delta^{15}N$ in biological tissues through an increased rate of ^{14}N excretion. Toxicant-mediated changes in protein metabolism may, therefore, influence the $\delta^{15}N$ of tissues. Our studies of snowy egret and largemouth bass exposed to dietary mercury in the laboratory, and bluegill, largemouth bass, yellow bullhead and brown bullhead collected from coal ash or mercury contaminated environments support this hypothesis. Snowy egret nestlings consuming environmentally realistic levels of mercury exhibited mercury-associated behavioral abnormalities and elevated relative $\delta^{15}N$ in the liver

and acid-soluble fraction of the liver. However, the metal binding peptides metallothionein (MT) and glutathione (GSH) did not differ between exposure groups. This challenges the expectation that biochemical responses are more sensitive than organism-level measures. Largemouth bass exposed to dietary mercury exhibited reduced relative gonad mass and increased δ^{15}N and GSH in liver acid soluble fraction. Bluegill inhabiting a coal ash-contaminated habitat had elevated hepatic MT, GSH, and δ^{15}N in the livers and acid-soluble fraction of livers. Similar associations were found between tissue δ^{15}N and contaminant levels in largemouth bass, yellow bullhead and brown bullhead collected from mercury-contaminated environments. These results show that shifts in δ^{15}N could serve as a marker for toxicant mediated shifts in the metabolism of protein nitrogen.

Towards a Completely New and Radically Different ERA Paradigm

Tannenbaum, Larry
U.S. Army Center for Health Promotion and Preventive Medicine, Environmental Health Risk Assessment Program

The case can be made that the current approach to characterizing ecological risk at historically contaminated properties leaves much to be desired, and further that what we think is needed in order to improve the science (e.g., good mechanistic understanding of metabolism and mode of action) does not aid in characterizing the probability of an adverse consequence. It would seem that for multiple decade-old contaminated sites, our energies should be going into characterizing any impacts that might have come about by this late date rather than endeavoring to predict future adverse consequences. A data-supported alternative viewpoint will be presented that suggests that traditional ecological risk assessments (ERA) do not provide the information being sought, and further, that ERAs are unnecessary overall for historically contaminated sites. The dire need for a shift from ERA to ecological *impact* assessment, whereby direct health assessment of the field-exposed receptor occurs, will be stressed. The only existing direct health status assessment method for the receptor in the field, the patent-pending RSA method (Rodent Sperm Analysis), will be introduced and explained with the aid of data from the method's applications to date.

Using Quantile Regression to Develop Stressor-Response Relationships for Community Metrics and Bedded Sediments from Field Data

Griffith, Michael B.[1]; Shaw-Allen, Patricia L.[1]; Cormier, Susan[2]; Chirhart, Joel[3]; Niemela, Scott[3]; Murzyn, Robert[3]
[1]U.S. EPA, Office of Research and Development, National Center for Environmental Assessment
[2]U.S. EPA, Office of Research and Development, National Risk Management Research Laboratory
[3]Minnesota Pollution Control Agency, Biological Monitoring Program

When community metrics calculated from field data are plotted against single stressors, the resulting data distribution may have a wedge shape. This distribution occurs because other stressors affect assemblage responses at individual sites in addition to the stressor of interest. Quantile regression can be used to fit a linear model to the upper bounds (e.g., 90th quantile) of a stressor-response relationship and estimate the expected response of an assemblage to the stressor of interest in the absence of other stressors. We used quantile regression to assess the relationships between various community metrics for macroinvertebrate and fish and %

fines, which was used as a measure of bedded sediments. The analysis used stream monitoring data compiled by MNPCA for the state of Minnesota. While also considering variation with stream size and between ecoregions, we found significant relationships between % fines and macroinvertebrate metrics, such as % abundance of very tolerant macroinvertebrates, clinger richness, POET richness, and % abundance of non-insects, and between % fines and fish metrics, such as darter, sculpin and madtom richness, % individuals that were simple lithophils, and % mass that were benthic invertevores. These relationships can be used to help assess the causes of biological impairment at other stream sites.

Multiple Land Use Effects on Upland Forest Vegetation of a Heterogeneous Military Site
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Fort Benning, a U.S. Army installation, lies within the fall-line sandhills, an ecotone between the Piedmont and Coastal Plain provinces. Vegetation on the installation has been shaped by interactions of land use with the heterogeneous environment. Lighter military use, dismounted infantry training cuts and tramples vegetation; heavier use, mechanized training compacts soil and crushes or uproots vegetation. Forest management by thinning and prescribed fire removes vegetation and alters soil resources. We conducted a field experiment to examine multiple land use effects on the ground layer of mixed pine-hardwoods stands on sandy and clayey soil. Over five years, stands in heavier and lighter military use compartments were subjected to an accelerated (2-year) or delayed (4-year) prescribed fire interval. NMDS ordination based on vegetation similarity separated sites with sandy and clayey soil. A second ‘degree of disturbance’ dimension separated sites with heavier military use and more frequent fire (less species-rich, more xeric vegetation) from those with lighter military use and less frequent fire. Differences due to fire developed after the second year. Grasses, legumes, and older stages of regenerating longleaf pine were most abundant in sites with relatively high historical (past 20 years) fire frequency and greater abundance of disturbance features, but fire caused mortality of tagged pine and hardwood seedlings. Further, nitrogen inputs did not offset nitrogen losses due to fire. Multiple land use presents a conundrum: heavier military use and frequent fire can maintain desired ground layer species composition, but may inhibit tree regeneration and result in nitrogen loss.

SESSION 2B: INTERNAL DOSE: THE ULTIMATE BUT ELUSIVE DETERMINANT OF RISK

Internal Dose and Response in Real-Time

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Rapid temporal fluctuations in exposure may occur in a number of situations such as accidents or other unexpected acute releases of airborne substances. Often risk assessments overlook temporal exposure patterns under simplifying assumptions such as the use of time-weighted averages (TWA) or cumulative exposures to assess risks. These simplifying assumptions may be appropriate in some situations, but not in others, depending on the nature of the compound and its toxic mode of action. We have developed and applied an exposure-dose-response model to study the neurotoxic effects caused in Long-Evans rats by acute exposure to trichloroethylene (TCE) vapors. The exposure-dose-response model includes a physiologically-based pharmacokinetic (PBPK) component that predicts momentary changes in the concentration of TCE in the brain during and after acute exposure. The momentary brain concentration is then compared to the amplitude of a pattern-elicited visual evoked potential (VEP) recorded from electrodes over the visual cortex. Each VEP waveform takes about 1 minute to record, and VEPs can be obtained repeatedly from rats during TCE exposure sessions. This enables assessment of the relationship between momentary brain concentration and neurophysiological function during and after exposure. The data suggest that the brain concentration of TCE at the moment of assessment is sufficient to describe changes in VEP amplitude, whereas cumulative exposure, area under the curve, or TWA was not. Momentary brain concentration may be an appropriate "dose metric" for use in assessing the risks of neurological impairment caused by acute exposure to TCE or other volatile organic compounds that cause similar acute neurological effects. (*This abstract does not reflect EPA policy.)*

Approaches to Determining Internal Dose in Inhalation Risk Assessment at the U.S. EPA

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In risk assessment, the respiratory tract is characterized as a complex target system. Inhalation exposure to toxic compounds (vapors and gases) may lead to both portal-of-entry and systemic effects. In addition, the estimated risk to humans is generally based on data from animal studies. Several dosimetric approaches exist to estimate the internal or delivered dose to a target-site from an inhalation exposure. These approaches vary in a continuum or hierarchy from default (e.g., set formula/equations) to optimal (e.g., CFD – computational fluid dynamics, PBPK – physiologically based pharmacokinetic models) depending upon the amount of chemical-specific and species-specific information available. One approach is outlined in the U.S. EPA's *RfC Methodology* (EPA/600/8-90/066F/October 1994). In this approach (data-limited), the ratio of the ventilation rate (V_E) to the surface area (SA) of the respiratory tract region of interest, of the animal to the human $[(V_E/SA)_A/(V_E/SA)_H]$, is used as an internal dose surrogate, assuming maximal absorption and uniform distribution. However, when more data are available, CFD, PBPK or CFD-PBPK hybrid dosimetry models are used. CFD (air-phase) models estimate the regional dose or flux (rate of transport) into tissue in both animals and humans, and suggest that absorption is localized and non-uniform. PBPK models incorporate data on tissue phase kinetics to estimate the tissue dose. Interspecies, hybrid CFD-PBPK models incorporate both air-phase and tissue-phase data to estimate tissue dose providing a

comprehensive description of a chemical's disposition. It is anticipated that an analysis of the results from the CFD, PBPK, and hybrid models will help inform the data-limited approaches.

Internal Dose Selection: Identification of Risk-Relevant Dose Metrics and the Impact on Extrapolation

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The Sapphire Group™

Summary

A key step in extrapolation using physiologically based pharmacokinetic (PBPK) models is the identification of a likely mode of action and appropriate dose metrics. For chemicals with an extensive pharmacokinetic database, chemical-specific animal-human scaling factors can be determined via physiologically based pharmacokinetic (PBPK) modeling. This issue was explored in a manner that can be generalized to other chemicals without an extensive database. PBPK-derived scaling factors were generated for chemicals where validated models are available. These PBPK-derived scaling factors differ among the various dose metrics; the results for the selected dose metrics were then analyzed for dose-dependence, species-dependence, and compared to theoretical scaling factors. The PBPK-derived interspecies scaling factors were generally consistent with theoretical scaling factors. Variation was largest for scaling factors based on a stable metabolite and was smallest for scaling factors based on amount metabolized. Furthermore, dose-dependence in allometric scaling is supported by this work. Examples of impact of the choice of dose metric on extrapolation results will be discussed.

Motivation

The assessment of risk to human health is typically performed by the comparison of a toxicity reference value to the measured or estimated exposure level. These toxicity reference values are frequently developed based on the results of controlled studies of animals or humans. The exposures in these studies may have both qualitative differences (e.g., route of exposure) and quantitative differences (e.g., exposure concentration) from the situation of interest for human health. Extrapolation is thus often required to derive toxicity reference values for human health risk assessment. The relevance of toxicity reference values can be refined by selection of an appropriate internal dose metric. If expressed appropriately, equivalent internal doses are expected to produce equivalent responses (toxicity) for a given species, independent of exposure scenario. The selection of an appropriate internal dose may be guided by an evaluation of the mode of action (MOA) and formulation of an internal dose metric appropriate to that MOA. Physiologically based pharmacokinetic (PBPK) models may be of use in calculating a variety of internal doses potentially relevant to different postulated MOAs.

Example 1—Theoretical Interspecies Scaling Factors for Internal Doses (Kirman *et al.*, 2003; Sweeney *et al.*, unpublished data)

The performance of allometric scaling of internal dose metrics using theoretical scaling factors (Clewell *et al.*, 2002) was tested using PBPK models for 20 chemicals (results with 12 chemicals were published as Kirman *et al.*, 2003; an extension of this work to 20 chemicals was performed by Sweeney *et al.*, unpublished). Model derived internal doses were computed for a wide range of concentrations. The PBPK model results generally supported the theoretically-derived scaling factors. An important finding is that above certain concentrations, the internal

dose-ratios were dependent on the exposure dose. These findings allow theoretical scaling factors to be used in place of PBPK-derived interspecies extrapolations when MOA assessment implicates certain internal dose metrics, but the available pharmacokinetic data are insufficient for PBPK model development.

Example 2—MOA-Dependent Interspecies Extrapolation for 2-Methoxyethanol (Sweeney *et al.*, 2001).

2-Methoxyethanol has been shown to have developmental effects in mice and rats. *In vivo* studies have correlated different developmental endpoints (exencephaly and digit malformation) with different dose metrics, namely peak or AUC for the metabolite 2-methoxyacetic acid (MAA). Interspecies extrapolation from a pregnant rat to a human occupational exposure was conducted using published PBPK models (Gargas *et al.*, 2000). The estimated human equivalent concentrations for the rat NOAEL were 12 ppm using daily blood AUC for MAA, 25 ppm using peak MAA (Sweeney *et al.*, 2001).

Example 3—MOA-Dependent Route-to-Route Extrapolation for 1,1,2-Trichloroethane (Sweeney *et al.*, unpublished data).

1,1,2-Trichloroethane (1,1,2-TCE) is designated as a Hazardous Air Pollutant. Route-to-route extrapolation has the potential to characterize risks via inhalation exposure for endpoints that have been studied by the oral dosing route. For example, in a cancer bioassay of 1,1,2-TCE, mice and rats were dosed by corn oil gavage (NCI, 1978). While we believe amount metabolized is the most appropriate dose metric for route-to-route extrapolation, we determined equivalent inhaled concentrations on the basis of five measures of internal dose (amount metabolized, and peak and AUC for parent compound in blood and liver). The “equivalent” inhalation concentrations varied as much as 100-fold between selected dose metrics, and the choice of the most “conservative” dose metric was dose-dependent.

Acknowledgements:
Financial support was received from Health Canada, Union Carbide, and the HAP Task Force.

Model code for the “scaling” project was supplied by Harvey Clewell, Tammie Covington, Rick Corely, Johannes, Filser, Clay Frederick, Sean Hays, Betty Hunt, Richard Luecke, Gina Pastino, David Plowchalk, Dick Reitz, Karla Thrall, and Chuck Timchalk.

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Assessment of Internal Dose from Hemoglobin Adducts
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The internal dose of a chemical or its reactive metabolite is generally expected to be more predictive of effects than exposure concentration. For reactive chemicals or reactive metabolites, adducts formed by reaction with macromolecules such as DNA or protein can provide a measure of internal dose. Adducts formed by reaction with blood proteins such as hemoglobin can provide a measure of internal dose, where the dose measured is integrated over the lifespan of the erythrocyte. With an understanding of exposure scenario, and measurement of reaction rate constants with hemoglobin, it is possible to estimate internal dose or AUC in blood. Recent studies with acrylamide, a carcinogenic and neurotoxic chemical produced in frying and baking, have investigated species differences, dose differences and route of exposure differences in the metabolism of acrylamide to glycidamide. Both acrylamide and glycidamide react with the *N*-terminal valine residue of hemoglobin. The adducts formed have been quantitated using a modified Edman degradation and LC-MS/MS. Adduct studies coordinated with characterization of metabolites in urine have indicated that the extent of metabolism of acrylamide to glycidamide is highest in mice, intermediate in rats and lowest in humans. The route of exposure influences the extent of metabolism via glycidamide. From the relative ratio of adducts, it appears that acrylamide is metabolized more slowly in humans, with less metabolism to glycidamide than in rodents.

Prospects and Limitations for the Use of Mode of Action Information and Biological Modeling to Characterize Internal Dose
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CIIT Centers for Health Research

Biological modeling, particularly physiologically based pharmacokinetic (PBPK) modeling, is increasingly being used in risk assessments to characterize measures of internal dose as a more biologically appropriate basis than administered dose for determining equivalent exposures across species and dose-route. However, the advantage of using internal dose comes at a cost of dealing with a number of uncertainties that can be ignored in a default approach. These uncertainties can be categorized into several tiers: (1) uncertainty regarding the mode of action for toxicity, (2) uncertainty regarding the appropriate measure of internal dose for a given mode of action, (3) uncertainty regarding the validity of the model structure and (4) uncertainty regarding the model parameters. Several case studies on applications of PBPK modeling in risk assessment will be presented to illustrate the nature of these uncertainties and to demonstrate approaches for dealing with them, ranging from decision tree analysis to hierarchical Bayesian analysis. The extension of biological modeling from PBPK dosimetry into the pharmacodynamic realm in order to model internal measures of interaction and response will also be discussed, and the associated additional challenges will be illustrated with examples. As biological modeling continues to deal with systems of ever-increasing complexity, including cell signal networks, the challenges associated with their evaluation will become more severe. Nevertheless, the value of biological modeling in risk assessment far outweighs the drawbacks.

SESSION 2C: GENOME BASED RISK ASSESSMENT ADVERSITY IN SENSITIVE POPULATIONS

A Mechanistic Approach to Identify Polymorphic Genes in Pathways Associated with Risk of Brain Cancer

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Enzymes in base excision (BER), nucleotide excision (NER), double strand break/ recombination (DSB/RR), mismatch (MMR), and direct-damage DNA repair pathways are important in the repair of diverse types of DNA damage. Polymorphisms in many of the genes encoding these enzymes have been identified as risk factors for environmentally and occupationally caused cancers. We evaluated the associations of polymorphisms in BER (*PARP1* V762A, *APEX* D148E, *MUTYH* Ex1+8A>C>G/T, *OGG1* S326C, *POLB* IVS11-235A>G, *XRCC1* R399Q, R280H, R194W), *LIG1* Ex2-24C>T, *PCNA* IVS1-124C>T), NER (*ERCC2* D312N, K751Q, *ERCC4* R415Q, *ERCC5* H1104D, *RAD23B* A249V, *LIG1* Ex2-24C>T, *PCNA* IVS1-124C>T), DSB/RR (*NBS1* Q185E, *RAD52* Y415stop, *XRCC2* R186H, *XRCC3* T241M, *XRCC4* N298S), MMR (*MLH1* I219V, *MSH2* G322D), and direct-damage repair (*MGMT* I143V, R178K, L84F) as risk factors for primary intracranial gliomas in the Upper Midwest Health Study, a population-based case-control study in rural residents of four states with high glioma incidence. Glioma cases (n= 798) were identified from hospitals, private physicians and registries. Control participants (n=1175) were stratified samples of licensed drivers and HCFA enrollees. Questionnaires elicited occupational and environmental exposures. DNA was obtained from 451 controls with no self-reported cancer and from 316 cases. TaqMan and MGB Eclipse methodology were used to characterize genotypes. In unadjusted analyses, a polymorphism in *PARP1* (V/V 67% of controls, 75% of cases, odds ratio (OR) 1.48, 95% confidence interval (CI) 1.07-2.04) had a statistically significant association with glioma, and polymorphisms in three other genes showed associations with glioma of borderline statistical significance: *RAD23B* A/V + V/V, 32% of controls, 38% of cases, OR 1.32, CI 0.97-1.78; *ERCC5* H/H, 61% of controls, 68% of cases, OR 1.33, CI 0.98-1.78; *XRCC4* N/N 74% of controls, 80% of cases, OR 1.37, CI 0.97-1.94. For each DNA repair pathway, multivariate logistic analyses included all polymorphisms in the pathway plus ever/never living on a farm and ever/never smoking, as surrogates for occupational and environmental exposure. Adjusting for these factors did not change odds ratios substantially. Our results should be confirmed in additional glioma case-control studies. Future analyses of our data will include assessing the risk of DNA repair polymorphisms under specific exposure conditions, such as exposures to pesticides, solvents and UV light.

Genetic Susceptibility Risk Factors for Polygenic Diseases

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Common diseases, such as asthma, Alzheimer's and cardiovascular diseases, are complex in nature, being variably influenced by physiological, life-style, environmental and genetic factors. As such, variations in individual genes that have the potential to affect a disease generally

possess relatively low or incomplete penetrance and consequently show low risk associations in epidemiological studies. A theoretical approach is presented, which allows estimation of the joint contribution of variations in individual genes to the risk of developing a disease. As an example, variants of 16 asthma susceptibility genes, including those associated with asthma mediators, atopy and chemical metabolism, were analyzed. A 6-fold increased risk of developing asthma for 20% in the general population occurred when only gene variants of asthma mediators were considered. With the addition of atopy variants, disease risk was almost doubled (OR=11). The odds ratio increased to 24 when all variants were included. The implications of these results for risk assessment under polygenic inheritance are discussed. Such a model can help establish the relative changes in risk associated with genetic-risk profiles in the population and help provide a framework for risk assessment.

The Role of Genetic Variations in Occupational Lung Diseases

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Inflammation is fundamental to the pathogenesis of many chronic lung diseases and common polymorphisms in inflammatory cytokine genes have been shown to influence individual susceptibility against various lung pathologies including cancer, asthma and COPD. In light of this, we studied cytokine gene polymorphisms, which may be associated with the development of dust-induced pulmonary fibrosis and the rate of accelerated decline in lung function. As has been found in the course of other chronic inflammatory diseases, several significant associations appeared between cytokine gene variants and disease progress and/or severity. We examined cytokine gene polymorphisms in ex-coal miners with silicosis and PMF and observed a strong association between severe silicosis and the TNFα -238 variant. An association between longitudinal rate of decline in lung function and genetic variations in cytokine genes were investigated in firefighters and the presence of IL-1β +3953, IL-1RA +2018 and TNFα -308 variants found associated with the decline rate of lung function as measured by FEV1. These findings suggest that specific variants of cytokine genes may influence individual susceptibility to occupational respiratory diseases.

Gender Differences in Proteomic Responses to III-V Semiconductor Particles

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The rapidly expanding use of III- V semiconductors such as gallium arsenide and indium arsenide during the last 20 years has resulted in both worker exposures to these materials during production processes and the need for recycling of outdated electronic devices (e - waste) in order to limit exposure to these toxic materials in the general population. Particles of GaAs and InAs are produced during the sawing, grinding and polishing of semiconductor wafers and in most countries producing these materials. Women constitute >50% of the production workforce so there is a pressing need to evaluate gender differences in susceptibility on a mechanistic basis for more precise risk assessment purposes. The present studies examined changes in gene expression patterns of renal tubule cells of male and female hamsters treated with 3-5 µM particles of either GaAs or InAs and examined at 10 or 30 days. Parallel *in vitro* studies using soluble salts of Ga, As or In alone, or on a mixture basis were conducted on

primary cultures of renal tubule cells derived from age matched male or female hamsters or archived human cells in order to compare proteomic response patterns by ^{35}S labeling and 2-dimensional gel electrophoresis. Overall, results of these studies demonstrated distinct differences in proteomic responsiveness between male and female renal tubule cells as a function of dose, semiconductor compound or metallic constituent and duration of exposure. In general, female derived cells showed greater proteomic responses to these treatments than males suggesting intrinsic gender differences (e.g., cellular programming) in cellular susceptibility. The implications of these data for improving risk assessment evaluations among workers and the general population will require further epidemiological studies. *(Supported in part by NIH R01 ES4979.)*

Risk Analysis for Benzene Using Molecular Biomarker Data

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For over two decades, scientists have been touting the importance of the application of biomarkers in reducing disease and protecting individuals from the harmful effects of exposure to occupational and/or environmental chemicals. However, few scientists apply stringent criteria to biological end points before proclaiming them biomarkers. While established guidelines for biomarker validation exist, methods for their implementation and case studies testing the methods are rare. This pilot study seeks to develop and demonstrate the use of a system for integrating complex and multifaceted data, validating biomarkers and incorporating the biomarkers into an occupational risk assessment. A survey of 59 occupational health safety professionals was conducted, and benzene was identified as an occupationally relevant, relatively data rich chemical. The structure for the biomarker database and decision rules were developed to organize the diverse types of data. A Bayesian network and regression analysis techniques were developed to test and validate (or discount) biomarkers along the entire exposure-disease continuum. The Bayesian network is used to analyzing the strength of the dependencies between exposure, the potential biomarkers and disease. The framework lays out an approach to consider a variety of biomarkers from the exposure-disease continuum for the enhancement of occupational risk assessment. Recommendations for utilizing biomarkers in general in risk assessment were developed and discussed. Future research will focus on refinement of the quantitative validation techniques and derivation of a revised, biomarker-based OEL for benzene.

SESSION 3A: HEAVY METALS OF EMERGING TOXICOLOGICAL CONCERN

Carcinogenic and Toxicological Effects of Embedded Tungsten and Tungsten Alloys

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Alloys of tungsten such as Tungsten/Nickel/
Cobalt (W/Ni/Co) and Tungsten/Nickel/Iron (W/Ni/Fe) are being developed as potential replacements for depleted uranium and as anti-personnel weapons. Tungsten (W) is also being considered as a replacement for lead in small caliber munitions. Two components of tungsten alloys, nickel and cobalt, are known carcinogens, and there is evidence that another component, iron, may increase the development of cancer. A preliminary study by Kalinich *et al.* (2005) demonstrated that rats implanted with W/Ni/Co developed aggressive, metastatic tumors, while rats implanted with Ni alone developed tumors but not metastases. These data suggest that W alloys may present a unique health hazard to soldiers wounded by W alloys. To better understand the progressive toxicological effects of these alloys as well as histological, biochemical and transcriptosome changes in tissues preceding or accompanying such events, we have implanted Fisher-344 rats with pellets of W, W/Ni/Co or W/Ni/Fe. Effects will be assessed 1-, 3-, 6-, 12- and 24-months post-implantation. Following three and six months of exposure, rats showed normal weight gain. There were no significant changes in organ/body weight ratios, biochemical or hematological parameters. Mild fibrous reactions were noted around pellets in all treatment groups. After six months rats implanted with W/Ni/Co began to develop tumors at the site of implantation.

Industrial Hygiene Aerosol Sampling

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Increasing emphasis is now placed on size selective sampling to monitor occupational exposure to aerosols. This presentation will provide a review of various Industrial Hygiene sampling devices with consideration to their advantages and disadvantages for the assessment of personal exposures to aerosol contaminants.

The Acute Effects of Tungsten Alloys (WA) on the Airway

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Penetrating munitions have traditionally been made with hard and heavy materials such as depleted uranium (DU). Because of the environmental and health concerns associated with DU, alternative materials including WA are now being developed and fielded. When WA munitions strike hard targets, aerosol clouds containing respirable particles as small as 0.2 µm are formed. Since military personnel near impact sites may be acutely exposed to high concentrations of aerosolized metals, the effects of WA on the airway were investigated. Male Sprague-Dawley rats were intratracheally instilled with either 20 mg of WA (91.1% W, 6.0% Ni, 2.9% Co) suspended in 250 µl of sterile saline or saline alone (control). After 24 hours (n=5/group), 48 hours (n=4/group) and 7 days (n=3/group), the animals were sacrificed; the lungs were lavaged with cold phosphate buffered saline and markers of inflammation and injury were measured in the lavage supernatant. Total protein (µg/ml) was significantly elevated in the

WA exposed rats at 24 hours (211.0±24.5) and 7 days (308.3±84.0) compared to controls (134.0±6.82 and 159.8±19.8, respectively). The WA treated animals also had significantly more lactate dehydrogenase 48 hours (0.12±0.01 µg/ml) and 7 days (0.39±0.12 µg/ml) after exposure compared to controls (0.07±0.002 µg/ml at 48 hours and 0.06±0.01 µg/ml at 7 days). Similarly, the concentrations of β-glucuronidase were significantly elevated to 130.6±22.1 µg/ml at 24 hours and 104.1±19.2 µg/ml at 7 days following WA exposure compared to controls (89.7±9.4 µg/ml at 24 hours and 64.6±8.6 µg/ml at 7 days). Significant amounts of tungsten (W) (121.1±24.5 ppm), nickel (8.4±1.4 ppm) and cobalt (1.3±0.2 ppm) were still present in the lung tissue 7 days after WA exposure compared to controls (0.02±0.002 ppm (W); below limit of detection (Ni) and (Co)). The detection of inflammation and metals in the lungs up to 7 days after WA exposure suggests that inhalation of munition particles may have not only acute but also long-term pulmonary effects.

Induction of Morphological Transformation and Global Disruption of Gene Expression in C3h/10t1/2 Mouse Embryo Cells by Specific Insoluble Nickel Compounds

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Exposure of refinery workers to nickel refinery dust containing soluble/insoluble nickel compounds correlated with increased incidences of respiratory cancer in the past. Nickel subsulfide and nickel oxide induce respiratory cancer in rats. C3H/10T1/2 (10T1/2) mouse embryo cells phagocytosed particles of insoluble nickel subsulfide and nickel oxide, which caused morphological transformation. We hypothesized carcinogenic insoluble Ni compounds induced global disruption of gene expression, resulting in morphological transformation. RAP-PCR mRNA differential display showed 130 genes were differentially expressed between non-transformed and four Ni-transformed 10T1/2 cell lines. There were higher steady-state levels of mRNA and protein from the ect-2 gene (rho GDP/GTP exchange factor), amplification of the ect-2 gene, higher steady-state levels of calnexin (molecular chaperone) mRNA and protein, and higher steady-state levels of Wdr 1 stress-inducible gene mRNA, in Ni- transformed cell lines. mRNAs from the vitamin D receptor interacting protein/thyroid hormone activating protein 80 (DRIP/TRAP-80) gene and two novel genes were silenced in Ni-transformed 10T1/2 cell lines. We propose that the uptake of Ni-containing particles releases intracellular Ni^{+2} ions, which (1) generate O^{2-}/$OH^{\cdot}$ radicals, capable of mutating/activating proto-oncogenes into oncogenes, and mutating/inactivating tumor suppressor genes, and (2) bind to histones, inducing chromatin condensation, methylation of promoter regions and silencing of tumor suppressor genes. Five Ni-induced alterations in proto-oncogenes and tumor suppressor genes disrupt expression of 120 genes, leading to cell transformation and carcinogenesis. *(Research supported by grant R01 ES03341/N. I. E. H. S., Contracts from NiPERA, and Training Grants 5T32 CA09320/N. C. I. to JRL (P. I.) and 5T32 AI078078/N.I.A.I.D.)*

Molecular Biomarkers for Evaluating Metal/Metalloid Interactions: An Overview

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Application of the modern tools of cell and molecular biology ("omic" technologies) have permitted detection and interpretation of early cellular responses following low to intermediate mixture doses of common toxic metals such as lead and cadmium and the metalloid arsenic. More recently, exposure to particles in the 3-5 µM range of the III-V semiconductors, gallium arsenide and indium arsenide, have also been evaluated using these approaches. Early, factorial design 10 week feeding studies in rats evaluated PbxCdxAs interactions using metabolomic disturbances of the heme biosynthetic pathway demonstrated clear additive interactions among these elements at "stressor dose levels" with regard to this pathway. Subsequent studies with drinking water exposures for 30, 90 and 180 days at empirically determined LOEL dose levels for these elemental combinations showed similar responses at 30 days, attenuated responses at 90 days and re-emergence of cellular responses at 180 days. These changes were associated with induction of several protective systems at 90 days, which appeared to be attenuated by 180 days. Intra-tracheal instillation studies of hamsters with 3-5 µM particles of GaAs or InAs produced both element–specific metabolomic disturbances of the heme biosynthetic pathway and proteomic alterations in protein expression patterns in renal tubule cells at 10 and 30 day time points. InAs produced marked inhibitory effects on protein expression patterns at 30 days relative to GaAs. These changes were correlated with GaAs and InAs–specific tubular proteinuria patterns which were most marked for InAs–treated animals at the 30 day time point. Confirmatory *in vitro* studies using primary cultures of hamster renal tubule cells also demonstrated the greater relative toxicity of the In+As combinations relative to Ga + As combinations. Overall, the results of these studies demonstrate the value of utilizing molecular "omic" approaches for both elucidating the relative toxicity of metallic toxins, alone or as mixture combinations, and providing basic mechanistic information on cell injury processes of value for interpreting results of toxicity studies for risk assessment purposes. *(Supported in part by NIH R01 ES 4979 and EPA STAR Grant #827169.)*

Toxicogenomics as a Tool for Identifying Biomarkers and Assessing Mechanisms of Action of Toxic Metals

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The advent of new genomics tools has provided a powerful new way to examine low level effects of chemicals on biological systems. It is clear from recent data that changes in gene expression at the genomic level represent one of the most sensitive measures of low level effects currently available, providing an endpoint that can detect responses at doses that are often several orders of magnitude lower than concentrations required to see a response with more traditional toxicological endpoints. However, because of this sensitivity, there has been considerable debate as to the toxicological significance of such low level changes in gene expression. In particular, it is not yet clear whether such low dose gene alterations are reflected in concomitant alterations in protein expression or function, cell phenotype or other downstream

effects that might be linked to actual biological responses at the physiological or pathophysiological level. Such concerns have so far precluded the application of toxicogenomics endpoints in the risk assessment process. It will, therefore, be important to determine the relationship between specific gene expression changes and downstream events, and to determine whether such changes reflect an adaptive response, a normal physiological response or a pathophysiological response. Nonetheless, toxicogenomics can provide a means to determine characteristic patterns of gene alteration that can be used to assess low level exposures, to identify potential biomarkers of exposure and effects, and to define a true “no effects” level. Toxicogenomics studies are also useful mechanistically both for hypothesis testing and for hypothesis generation, but must ultimately be linked to other downstream endpoints in order to determine their toxicological significance.

SESSION 3B: ISSUES AND APPLICATION OF MODE OF ACTION IN CANCER RISK ASSESSMENT

The Use of Carcinogenic Mode of Action Data in Human Health Risk Assessment

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U.S. EPA's recently released *Guidelines for Carcinogen Risk Assessment* and *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure* make greater use of the increased scientific understanding of the biological mechanisms leading to cancer following environmental exposures. The Guidelines suggest that mode of action (MOA) data, when available and of sufficient quality, can be useful for understanding the potency of a chemical, its potential effects at low doses, whether findings in animals are relevant to humans, and which populations or lifestages may be particularly susceptible. The *Supplemental Guidance* notes that childhood may be a lifestage of greater susceptibility for a number of reasons, such as rapid growth and development that occurs prenatally and after birth, differences related to an immature metabolic system, and differences in diet and behavior patterns that may increase exposure. U.S. EPA's Integrated Risk Information System (IRIS) Program is considering mode of action data in evaluating the carcinogenicity of environmental chemicals. Key implementation issues focus on the weight-of-evidence for a hypothesized MOA, lifestage issues related to carcinogenesis, and data needs for determining a mutagenic mode of carcinogenic action. *(The views expressed in this abstract are those of the author and do not necessarily reflect the views of U.S. EPA.)*

A Framework for Human Relevance Analysis of Information on Carcinogenic Modes of Action

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A precedent-setting legislative mandate to establish priorities for assessment based on systematic consideration of all of the approximately 23,000 Existing Chemicals in Canada has required the development and refinement of methodology in a number of important areas. For priority setting, this has included development of a transparent framework to address weight of evidence for hazard for cancer and genotoxicity, based on often limited data and the considered output of a range of expert and statistically-based modeling systems. For the fuller risk assessment components of the program (i.e., screening and Priority Substances assessments), methodology includes frameworks to transparently consider the weight of evidence for hypothesized modes of action.

Specifically considered is a framework for human relevance for cancer developed by the International Life Sciences Institute Risk Sciences Institute (ILSI RSI), in work sponsored by Health Canada and the U.S. Environmental Protection Agency (U.S. EPA). This framework is based on consideration of the likelihood of occurrence in humans of observed tumors in experimental species based on qualitative and quantitative analysis of concordance for key events in the hypothesized mode of induction. The framework includes explicit description of confidence in the evaluation, identification of specific data gaps and the implications for risk assessment. The framework, which was developed and refined through its application in case studies for principally non-DNA reactive carcinogens, has more recently been extended to DNA reactive carcinogens, noncancer endpoints and different life stages.

Use of Genetic Toxicology Data in Establishing a Carcinogenic Mode of Action
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U.S. EPA, Office of Water

In 2005 EPA published revised Guidelines for Carcinogen Risk Assessment and "Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens." The Supplemental Guidance will be revised as the science supports. This document evaluates evidence for differences in tumor outcome based on the lifestage at which carcinogen exposure occurs. Based on this evaluation, recommendations are made to consider likelihood of increased cancer susceptibility when exposure is post-natal through adolescence; in particular numerical adjustment of the slope factor is to be done for mutagenic carcinogens when data are insufficient for calculation of specific early-life potency. Identification of a mutagenic MOA for a chemical carcinogen has, thus, become of increased importance. Definitions of "genotoxic" and "mutagenic" are numerous, often interchangeable and sometimes contradictory. Moreover, the operational definition of mutagenic is set by results of a variety of test systems. New tests continue to develop, and interpretation of results of individual assays, or batteries of assays, change with time. Determination of mutagenic MOA can't be done with a static checklist; rather it must be a weight-of-evidence analysis, dependent on factors such as the number and types of tests or measurements, as well as consistency and coherence of results. Transparency and consistency in these judgments will be promoted by using the principles underlying the MOA framework in the Cancer Guidelines and agreed-upon interpretations of genotoxicity data.

Application of Mode of Action Data in Risk Assessment - Inorganic Arsenic
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Arsenic is classified as a human carcinogen. Exposure to arsenic from drinking water is associated with the development of skin as well as internal cancers (e.g., lung, bladder cancers) in human populations. The majority of the epidemiological studies conducted so far are from regions where arsenic concentrations in drinking water are considerably high (~100 ppb and above). This poses a challenge in estimating the cancer risk to humans at low doses. Understanding the mode of action data for arsenic is essential for extrapolating cancer risk at low doses. Although inorganic arsenic is established as a human carcinogen, rodents are generally nonresponsive to the tumorigenic effects of inorganic arsenic. Further, the toxicity of arsenic depends on the formation and distribution of metabolites and the valence state of the arsenic species in the target tissues. Although the major metabolic products, MMA^{v} (monomethyl arsenic acid) and DMA^{v} (dimethylarsenic acid), are readily excreted in urine, the methylation of inorganic arsenic is not entirely a detoxification process. Arsenic is not mutagenic but several possible modes of action such as formation of chromosomal aberrations, sister chromatic exchange, induction of micronuclei, oxidative stress, alteration in gene expression, inhibition of DNA repair enzymes, and alteration of signal transduction pathways have been suggested. Despite the multiple modes of action possible for arsenic, the existing evidence is not considered adequate to deviate from the linear dose response modeling in estimating the cancer risk quantitatively to humans.

Recent Insights into Benzene's Mode of Action and Their Implications for Cancer Risk Assessment and Low Dose Extrapolation

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Benzene, an important industrial chemical and common environmental pollutant, is an established human and animal carcinogen. While many of the mechanisms underlying its toxic and carcinogenic effects remain unknown, a number of recent studies are providing valuable insights into the metabolites and mechanisms that are likely to play important roles in its hematopoietic effects. The objective of this presentation will be to review prominent mechanisms that have been proposed to underlie benzene-induced leukemia, such as DNA binding, topoisomerase II inhibition, oxidative stress, bone marrow toxicity and altered differentiation, and then discuss the contributions of recent studies in providing insights into benzene's mode of action and in estimating its effects at low doses. The possible contribution of multiple mechanisms in benzene's carcinogenic effects will also be discussed.

Probing the Mode of Action of Tumorigenic Conazoles Using Traditional and Toxicogenomic Approaches

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An approach is described to uncovering mode(s) of action (MOA) for mouse hepatotumorigenic conazoles using traditional toxicological and toxicogenomic approaches and employing carcinogen/non-carcinogen activity pairs. Male CD-1 mice were treated in the feed for 4, 30 and 90 days with triadimefon (0, 100, 500 or 1800 ppm), propiconazole (0,100, 500 or 2500 ppm), or myclobutanil (0, 100, 500 or 2000 ppm). Both triadimefon and propiconazole are known to induce hepatic adenomas/carcinomas in male mice, while myclobutanil is inactive as a mouse liver tumorigen. In conjunction with mouse liver enzyme, histopathologic and other analyses, genomic expression profiles of the mouse liver samples were analyzed for patterns that would potentially reveal the MOAs of the different tumorigenic conazoles. Our analytical approach was first to identify differentially expressed genes, perform cluster analyses and identify those pathways that were altered for each conazole. Then, pathways associated with the tumorigenic conazoles were uncovered using the differential expression changes: (a) between the tumorigenic and non-tumorigenic conazoles, (b) between the tumorigenic and non-tumorigenic doses and (c) pathways that were more strongly associated with the tumorigenic conazoles. These analyses were supplemented with further explorations into time and dose relationships as well as an integration of altered pathways into interactive groups. (*This abstract does not reflect EPA policy.)*

SESSION 3C: ASSESSING THE RISK OF HEARING LOSS FROM NOISE AND CHEMICALS

The Solvent Effects on the Auditory Efferent Pathway

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It is now admitted that aromatic solvents can cause impairments to the auditory system. Solvent-induced hearing loss is an example of a selective organ-directed toxicity. The fact that the outer hair cells are more sensitive to solvents than the inner hair cells can be considered as a major characteristic of the solvent ototoxicity. Moreover, the potentiation of the noise effects by a solvent exposure is also a characteristic that is of critical importance in terms of occupational safety. So, the present experiment was designed to study the risks encountered by subjects exposed to both hearing stressors: solvent and noise. Anaesthetized rats were equipped with an electrode placed on the round window of the cochlea to record the cochlear microphonic potential (CMP), which is a reliable electrical indicator of the outer hair cells function, whereas aromatic solvent emboles (vehicle: intralipid) were directly injected into the carotid to avoid the likely ototoxic action of the metabolites. Contrary to our expectations, the amplitude of the CMP increased following the solvent injections and the increase in the amplitude was correlated to the noise intensity. Furthermore, the increase in the amplitude of the CMP was also reproduced by the injection of acetylcholinergic antagonists: atropine and α-bungarotoxin for instance. The results of this study let us think that the aromatic solvents could play a major role at the level of the cerebral trunk by inhibiting the auditory efferent system and more specifically the olivo-cochlear efferent system. Finally, the hypothesis that solvents may target ligand -gated ion channels will be discussed in the presentation.

Ototoxic Stress: Styrene-induced Hearing Loss

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Styrene is a neurotoxic organic compound used industrially worldwide, and is classified as a possible carcinogen. Styrene 7,8-oxide is the main metabolite of styrene, which induces apoptosis with caspase activation. It is known that styrene exposure results in hearing dysfunction in the mid-frequency range and causes death of the hair cells and supporting cells. However, the cell death pathway is still unclear. In this experiment, rats were exposed to styrene by gavage (once a day). The dosages used were 200, 300, 400 and 800 mg/kg body weight in oil. The blood styrene level reached the highest level within 30 min (21 µg/g for 800 mg/kg dosage), which lasted for about 6 hours. The styrene level decreased to half of the highest level within about 8 hours, and no styrene remained in the blood 24 hours after the treatment. A dose-dependent hearing loss in the middle-frequency range was observed, with related hair cell loss. A significant threshold shift (8.9 dB) was observed after 300 mg styrene treatment (5 days/week for 3 weeks) which increased to 30.3 dB at an 800mg/kg dosage. Although the styrene treatment with 200 mg/kg dosage did not cause a significant hearing loss, it caused about 5% outer hair cell (OHC) loss. OHC death started about 3 days after styrene treatment at 800 mg/kg dosage. Apoptosis was seen in the 3rd row of Deiters' cells prior to OHC death. OHC death resulted from apoptosis with caspase activation. It appeared that both death receptor-mediated and mitochondria-mediated apoptotic pathways are involved in styrene-induced OHC death. Interestingly, treatment with N-L-acetylcysteine (NAC), a free radical scavenger, protected against the styrene-induced hearing loss and hair cell loss.
[Research supported by grant 1R01OH008113-01A1.]

Multi-disciplinary Techniques in the Assessment of Neurotoxic Effects of Solvents on the Central Auditory System

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Several organic solvents that we encounter in our daily lives through inhalation, ingestion or skin absorption are known to injure the nervous system by damaging cortical as well as sub-cortical regions of the brain. Although heavy exposures to solvents have been identified as causing solvent encephalopathy, low-level exposures are not so well characterized. A fairly large volume of the nervous system is devoted to the processing of auditory information, so there is a relatively high probability that injury to the nervous system can be identified, characterized and monitored using audiological tests. However, information gathered from these tests does not provide the exact nature of the impairment caused by neurotoxic solvents, nor does it provide dose-response relationships. *In vitro* techniques, using cultured central auditory neuronal networks growing on microelectrode arrays, can be used as a model system to study the electrophysiological and morphological damage caused by solvents and their mixtures. Various audiological techniques as well as techniques using the *in vitro* biosensor system will be briefly described in this session.

Chemical Exposure as a Risk Factor for Hearing Loss: Implications for Occupational Health

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Research conducted over the last two decades has brought attention to the interaction between noise and chemicals in the workplace as a cause for hearing disorders. Since then, several research labs have become involved in investigating the ototoxic properties of agents such as toluene, styrene, xylenes, ethyl benzene, n-hexane, trichloroethylene, stoddard solvent, carbon monoxide, hydrogen cyanide and lead. Reports confirmed earlier observations that some chemicals interact synergistically with noise or potentiate its effects on the auditory system. Studies have shown that chemicals reached the inner ear through the blood stream, were found in the inner ear fluids and have caused damage to some of the inner ear structures and functions. Although noise is particularly damaging to the cochlea, industrial chemicals tend to affect both the cochlear structures and the central auditory system. This compound action may profoundly impact a worker's particular hearing loss because not only will the detection of sounds be impaired but also the discrimination of sounds may be affected (i.e., not only will sounds be perceived as less loud but also as more distorted). In light of the many chemicals that are used in the work place and evidence that they may affect hearing, numerous populations are being underserved with regard to the prevention of hearing loss. The new evidence has prompted the proposal of new guidelines and standards on the prevention of hearing loss from ototoxic agents. This presentation will review the current knowledge of chemical ototoxicity and discuss research needs regarding hearing loss prevention.

U.S. Army CHPPM Guidelines for Preventing Hearing Loss from Chemical Exposures

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In the year 2003, the U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) published a Fact Sheet, Occupational Ototoxins (Ear Poisons) and Hearing Loss. This fact sheet was prepared to answer the repeated inquiries from U.S. Army industrial hygienists seeking guidance. The literature was surveyed to ascertain what chemicals were considered to be potentially ototoxic, and which of these could be dermally absorbed in significant amounts if the dermal pathway was not protected adequately. Then, the U.S. Army database for occupational exposures was queried to determine which of the ototoxins were being used within the U.S. Army, as well as where and at what frequency. The fact sheet recommends that yearly audiograms be conducted on workers whose exposures (without regard to respiratory protection worn) are at 50% or more of the Occupational Exposure Limit (more stringent of the ACGIH TLV or OSHA PEL) for the specified ototoxic substances in question, regardless of the noise level. It recommends that if there are dermal exposures to specified ototoxic substances and such exposures may result in a systemic dose equivalent to 50% or more of the OEL, yearly audiograms are also recommended. The nature and level of the ototoxin is recommended to be included in the comment sections of DD 2215, Reference Audiogram and DD 2216, Hearing Conservation Data. The Ototoxin Fact Sheet is available on the world wide web at: http://chppm-www.apgea.army.mil/documents/FACT/51-002-0903.pdf, or at the main CHPPM web site: http://chppm-www.apgea.army.mil/, under USACHPPM Resources, Fact Sheets.

LUNCHEON SESSION: AN OVERVIEW OF FEDERAL, STATE AND LOCAL PUBLIC HEALTH INVESTIGATIONS OF TRICHLOROETHYLENE CONTAMINATION SITES

An Overview of Federal, State and Local Public Health Investigations of Trichloroethylene Contamination Sites

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TCE (trichloroethylene: CAS # 79-01-6) has been found at over 800 National Priorities List (NPL) sites, but it is unknown how many sites have been evaluated. It is suspected that the number of sites contaminated with TCE is significantly greater than this. Regardless, its presence in drinking water at greater than the EPA MCL of 5 ppb in many communities in the U.S. has become an increasingly prominent public health issue as has its association with various adverse outcomes. Exposure to TCE has been assigned blame for a number of adversities including cardiac developmental abnormalities, to gestational age size/weight deficits to kidney cancers. Drinking water is not the only exposure pathway of interest, however. At least six senators have petitioned EPA to issue a health protective interim standard for TCE vapor intrusion, and states are struggling with establishing indoor air action concentrations. ATSDR has conducted public health assessments, health consultations, and epidemiologic studies at multiple sites contaminated with TCE examining all pathways of exposure and multiple endpoints or health outcomes. Whether it is DOD, EPA, ATSDR, or state health departments conducting the investigations, managing the risks associated with TCE exposure has been and continues to be a daunting task.

SESSION 4A: MODE OF ACTION IN METALS IN RISK ASSESSMENT: CONFOUNDERS AND IMPLICATIONS OF ARSENIC AND ORGANOTIN COMPOUNDS

Arsenic Cancer Risk Assessment

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The assessment of the cancer risk to humans from exposure to arsenic through the drinking water route has proven to be a complex problem. This complexity is the result of the fact that it appears that arsenic has several plausible modes-of-action and also that there is not an appropriate animal model for arsenic carcinogenicity that allows for study of these various modes-of-action with tumors as an ultimate endpoint. In addition, arsenic induces a range of different tumor types, each of which could have different modes of formation. The modes-of-action include genotoxicity (probably from oxidative DNA damage) abrogations of DNA repair and cell cycle checkpoints, cytotoxicity and regenerative cell proliferation, alterations in genomic methylation patterns, and alterations in gene expression. Which of these is operational under specific circumstances remains to be determined. It is also not known if any one of these modes-of-action is more likely at low concentrations of arsenic typical of most drinking water sources. The area is ripe for study especially employing the new genomic techniques and accompanying computational tools.

Another aspect of there being multiple modes-of-action is that there are predicted to be a number of confounders of response that include genetic, epigenetic and environmental factors. For example, polymorphisms in DNA repair genes, metabolizing enzymes or cell cycle genes could influence the magnitude of a carcinogenic response. In addition, dietary factors have also been proposed as confounders of tumor responses. All of these possible confounders need to be considered when conducting a cancer risk assessment.

The emphasis of the presentation will be on cancer risk assessment, but there are other adverse health outcomes that have been associated with arsenic exposure, including gastroenteritis, neurological manifestations, vascular changes and diabetes. These noncancer effects need to be considered if the total health detriment from arsenic exposure is estimated. Again, the assessment of risk is complicated by there being multiple endpoints and multiple modes-of-action. *(This abstract does not necessarily reflect U.S. EPA policy.)*

Arsenic Carcinogenicity - Mode of Action

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Atmospheric release of arsenic occurs from both natural and anthropogenic sources. While annual global natural emissions are estimated around 8000 tons, anthropogenic emissions are about three times higher. Arsenic is an environmental carcinogen of global public health concern affecting millions of people through contaminated drinking water supplies. In fact, it is one of the earliest identified human carcinogens, when medical treatment for Psoriasis with Fowlers solution (1% potassium arsenite) was found to result in excess of skin cancer. Epidemiological data have shown that chronic exposure of humans to inorganic arsenical compounds is associated with liver injury, peripheral neuropathy, and an increased incidence of cancer of the lung, skin, bladder and liver.

Arsenic is not a recognized mutagen in bacteria. Earlier studies using hprt assay in Chinese hamster cells and *E. coli* gpt locus in transgenic G12 cells suggested arsenite to be a weak mutagen. However, recent studies utilizing the A_L human-hamster hybrid cell assays demonstrated arsenite as a potent mutagen. Biomethylation is a major detoxification pathway for inorganic arsenicals (iAs) in humans and in most animal species. Methylation requires preceding metabolic reduction of pentavalent arsenic to trivalent arsenic. Subsequent to metabolic reduction, arsenic is methylated via glutathione conjugation in two steps to monomethylarsonic acid (MMA^V), and dimethyl arsenic acid (DMA^V). A second methylation step results in trivalent methyl derivatives of arsenic MMA^{III} and DMA^{III}. Methylation process enhances the toxicity of arsenicals and trivalent methyl species are in fact found to be potent genotoxicants. Based on the accumulated scientific evidence so far, nine different modes of action have been proposed for arsenic carcinogenicity including chromosomal abnormalities, oxidative stress, altered expression of growth factors, altered cell proliferation and altered DNA repair. It is now, strongly believed that these mechanisms might not operate in isolation and that arsenic induced-carcinogenesis is the result of more than one of these pathways operating at any given instance.

Several confounding interactions by environmental, dietary and genetic factors such as Ultra Violet (UV) radiation, dietary selenium, zinc, polymorphisms in glutathione-s-transferase (GST) gene, and inter individual difference in methylation pattern have been found to modulate arsenic toxicity or carcinogenicity. Epidemiologic studies reported increased incidence of skin cancer in arsenic endemic areas of the world suggesting a potential confounding role for UV radiation. Enhanced genotoxicity of UV upon arsenical exposure has been demonstrated in both prokaryote and *in vitro* and *in vivo* systems. Genotoxicity associated with UV-B exposure is mediated by DNA damage. To further understand the potential interaction between UV-B and arsenic, which may result in acceleration of cell cycle and proliferation of cells with compromised DNA repair and ultimate skin cancer.

To explore this hypothesis, we have hypothesized that arsenicals may override UV-induced growth arrest and cause proliferation of unrepaired/ misrepaired cells. Towards understanding such interactions at a molecular level, we have utilized an *in vitro* primary keratinocyte cell culture system (consisting of normal human epidermal keratinocyte, NHEK) exposed to UV-B (100 mJ) to induce growth arrest and DNA damage and simultaneously obtain a homogeneous population of cells in G0 phase. These cells were exposed to non-cytotoxic concentrations of inorganic (iAs), (0-12 µM) and two of its trivalent methyl metabolites, MMA^{III} (1-2 µM) and DMA^{III} (1-3 µM). Simultaneously a second set of cells were exposed to arsenicals without prior UV exposure

Results obtained from these studies elegantly demonstrated UV, arsenic interactions in this target cell model. Arsenicals induced a concentration dependent increase in proliferation of NHEK cells with or without prior UV exposure when analyzed at 24h post exposure. In post UV-exposed cells significant cell proliferation was observed even at a low concentration 0.6 µM compared to cells without prior UV exposure, where proliferation was observed between 2-6 µM of iAs. Peak proliferation was observed with iAs at 6.0 µM, while 12 µM, arsenic was cytotoxic. DMAS III induced cell proliferation in UV treated cells reached its peak (26%) at 0.8 µM. The concentration of MMAsIII required to cause significant cell proliferation also varied based on prior (0.4-1.0 µM) or post (0.5 and 0.8 µM) UV exposure. Flowcytometric analysis for cell cycle distribution revealed that in post-UV exposures arsenicals predominantly arrested cells at G2/M at 24h.

Analyses of a battery of cell cycle proteins such as Cyclin D1, cdk5, PCNA, Cdc 25A and Cdc25C, revealed arsenical-specific alterations in expression, indicating their role in cell cycle abrogation or DNA damage repair at G2 phase of the cell cycle. The results obtained from these studies also clearly demonstrated involvement of epidermal growth factor (EGF) in NHEK cell proliferation. Further the observation of JNK mediated signaling events in proliferation of NHEK cells with UV+ arsenical exposure and ERK in the cells without prior UV exposure demonstrated selective activation of downstream signaling pathways in arsenicals-induced proliferation. This is the first such demonstration of selective activation of down stream signaling pathways in the confounding interaction of UV and arsenicals in keratinocyte model. The results obtained in this study indicated the confounding interactions between UV and arsenicals in arsenical driven proliferation of UV damaged cells that may be mediated by more than one of the modes of action. These studies also demonstrate, the need and utility of such relevant target based cell systems to aid in the potential development of biomarkers to understand the confounding role of UV in endemic areas of arsenic exposure world wide. (*This abstract does not reflect EPA policy.*)

On the Mechanism of Arsenic-Associated Skin Cancer

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Inorganic arsenic in drinking water has been associated with skin cancers in epidemiological studies in a number of countries such as Taiwan, China, Chile, Argentina, India, Bangladesh, and Mexico. This association has not been seen in the United States. In addition, inorganic arsenic alone in drinking water does not cause skin cancers in animals. Arsenic is metabolized in the liver by sequential methylations and reductions as follows: arsenate -> arsenite -> MMA^{V} -> MMA^{III} -> DMA^{V} -> DMA^{III}. Trivalent arsenic compounds are more toxic than the corresponding pentavalent ones. Earlier work from this laboratory demonstrated that arsenite (the likely agent in water responsible for increasing human cancers) is not mutagenic, but is able to act as a comutagen (enhance mutagenesis) by genotoxic agents. Analysis of reports of chromosome aberrations (CA) induced by trivalent arsenicals reveals that the concentrations of arsenicals that gave significant increases in CA were toxic. This might explain the lack of mutagenicity by arsenicals at loci that can detect other clastogens. The lack of mutagenicity and the lack of carcinogenicity in standard bioassays calls into question whether arsenic should be considered a genotoxic carcinogen.

To test the hypothesis that arsenite might be a cocarcinogen (since it is a comutagen), we recently showed that a concentration as low as 1.25 mg/L sodium arsenite (a concentration found in some human drinking water) was able to enhance the tumorigenicity of solar UV (UVA + UVB) irradiation in mice. The tumors were almost all squamous cell carcinomas, appeared earlier and were more malignant than in mice exposed to UV alone. No tumors were seen on skin or any other organ of mice receiving arsenite alone up to 10 mg/L. This data suggests that arsenic in drinking water may need a carcinogenic partner, such as sunlight, in the induction of skin cancers. Rumors to the contrary, the locations of skin tumors in humans exposed to arsenic in drinking water are consistent with sun exposure as a factor.

There are a number of possible mechanisms for cocarcinogenesis by arsenite, including: inhibition of DNA repair (which occurs indirectly, not by enzyme inhibition), increased cell proliferation (which occurs in mouse skin by arsenite alone), increased oxidant signaling, altered

DNA methylation (associated with arsenite-induced malignant transformation), aneuploidy (also associated with transformation), inhibition of apoptosis and genomic instability. In support of the latter, we have demonstrated that cells grown in low (0.1 µM) concentrations of arsenite have normal mutation rates for about 15 generations, and then suddenly generate increased mutants, a phenomenon we call “delayed mutagenesis”. Delayed mutagenesis can be blocked by selenium compounds. Vitamin E and p-XSC (a synthetic organoselenium compound) each blocked the arsenite-induced enhancement of UV-induced skin cancers in mice, but the effect of the selenium compound was specific against arsenite, because vitamin E reduced UV (alone)-induced carcinogenesis, whereas p-XSC did not.

Malnutrition has been shown to increase the risk of arsenicosis in underdeveloped countries. Besides selenium, low dietary folate is expected to increase arsenic susceptibility by reducing arsenic methylation (which will slow excretion) as well as interfering with DNA methylation and causing uracil incorporation into DNA (a genotoxic event). It is of interest that malnutrition resulting in low blood sugar is expected to increase the uptake of arsenite into cells via hexose permeases and aquaglyceroporins. The differences between the U.S. and other arsenic-exposed populations with regard to skin cancers might be explained by the lower levels of arsenic in the U.S. drinking water, less sun exposure, less exposure to other carcinogens, better nutrition, and genetic susceptibility differences. It is possible that arsenic compounds increase lung and bladder cancers by enhancing the effects of other environmental agents, similar to the enhancement of skin cancer induced by solar UV.

Interpretation of Biomonitoring Studies to Assess Exposure and Risk of Inorganic Arsenic: Confounding by Other Sources of Arsenic

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Gradient Corporation

Arsenic can exist in the environment in a number of different forms, each form with its own unique toxicology. Inorganic arsenic (InAs), the most toxic of the different arsenic species found in the environment, is a well-established, lung, bladder, and skin carcinogen. Additionally, InAs has been associated with several noncancer effects, including peripheral vascular disease (PVD) and diabetes. In contrast, the organic arsenic compounds, including the pentavalent species, monomethylarsenic acid (MMA^{V}) and dimethylarsenic acid (DMA^{V}), are significantly less toxic and have not been associated with any adverse health effect at environmental exposures. Potential exposure to arsenic compounds, both inorganic and organic, commonly occurs through the ingestion of food and water.

A great deal of research has gone into understanding the mechanism of arsenic toxicity and its potential dependence on metabolism. Upon ingestion, InAs is taken into cells and extensively metabolized by sequential oxidation and reduction reactions to trivalent and pentavalent forms of monomethyarsenic acid (MMA^{V}), dimethylarsenic acid (DMA^{V}), and (in certain animal species) trimethylarsine oxide (TMAO). Excretion is mainly into the urine. In general, InAs accounts for only 10% of urinary arsenic metabolites; the rest of the arsenic detected in urine in humans is mainly in InAs's metabolized forms: MMA (~20%) and DMA (~70%). Recent studies have demonstrated the intracellular metabolism of InAs also generates the methylated trivalent species, monomethylarsonous acid and dimethylarsinous acid (MMA^{III} and DMA^{III}). These metabolic intermediates are generally considered more cytotoxic than InAs and may be involved, in part, in inorganic arsenic-induced carcinogenesis. Unlike inorganic arsenic compounds, MMA^{V} and DMA^{V}, when ingested, do not undergo extensive metabolism, and are

excreted mainly unchanged into the urine. Due to the limited metabolism of methylated arsenic compounds in humans, the ingestion of MMA^V or DMA^V does not produce meaningful amounts of cytotoxic intermediates (MMA^{III} and DMA^{III}). Thus, by virtue of their metabolism, the different arsenic species are associated with significantly different toxicities.

Human biomonitoring studies have commonly measured urinary metabolites to quantify arsenic exposure and, more recently, to investigate the role of metabolites in the formation of specific tumors and etiology of certain diseases. For example, several studies have demonstrated that an increased secondary methylation index (SMI) (i.e., a higher ratio of DMA/MMA[1]) is associated with a lower risk of skin and bladder cancer, as well as PVD. Biomonitoring studies relating arsenic metabolic ratios to disease can provide potentially important information regarding how the presence of urinary arsenic metabolites relates to health risks from inorganic arsenic, i.e., this information can be useful in understanding differences in responsiveness to ingested inorganic arsenic and in identifying the relevant toxicologic moieties. However, measuring the exposure to InAs through the examination of urinary metabolites can be confounded by environmental exposures to the methylated compounds because direct ingestion of these less toxic methylated pentavalent arsenic species will increase urinary concentrations of MMA and DMA. In areas where exposure to InAs in drinking water is high, the confounding effects of exposure to the methylated arsenic compounds in food will not likely be significant. However, in areas where InAs exposures are relatively low, such as most areas in the U.S., exogenous ingestion of MMA^V and DMA^V in foods could represent a large portion of the arsenic detected in urine. Not properly accounting for methylated arsenic in the diet could obscure efforts to quantify InAs exposure and characterize the potential relationship between InAs metabolism and disease.

Based on a diet consisting of rice and fish in a "low" vs. "high" InAs area, we conducted a hypothetical analysis to quantify the relative contribution of ingested InAs and DMA to urine arsenic. Low arsenic areas were defined as areas dependent on drinking water sources containing 1 μg/L InAs and high arsenic areas were assumed to have an InAs drinking water concentration of 100 μg/L. We determined the relative contribution of InAs and DMA to urinary concentrations using two different dietary estimates of arsenic. One exposure scenario was based on an average daily dose (ADD) of selected seafood and white rice in the U.S. diet, and the other on single meal of shrimp, clams and white rice. Based on an ADD of rice and seafood in low arsenic areas, DMA^V from the diet contributed 75 % of the DMA^V in urine, whereas in high arsenic areas, it only contributed 6%. Assessing these relative contributions after a single meal of seafood and rice produced even more disproportionate results in the low arsenic areas. In this scenario, 95% of the DMA in urine was from DMA^V in the diet. DMA^V-derived dietary arsenic also had a marked effect on the calculated SMIs, which were about 4-6 fold higher (depending on dietary exposure scenarios) in low arsenic areas compared to high arsenic areas.

Thus, accurately determining exposure to arsenic and its possible relationship to disease require consideration not only of all potential sources of arsenic, but of the form of arsenic ingested. A failure to account for urinary metabolites derived from ingestion of forms of arsenic associated with relatively low toxicity will mischaracterize InAs exposure and may lead to overestimates of risk. The most scientifically supportable risk assessment for InAs will

[1] Methylated arsenic metabolites in urine are usually measured as a combination of the trivalent and pentavalent species. For example, the concentration of DMA is the sum of DMA^V and DMA^{III} urinary levels.

recognize the unique metabolism and toxicity of individual arsenic compounds and will evaluate each arsenic compound separately.

Modeling Human Exposure to Organotins in Tap Water via Migration from PVC and CPVC Piping

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Polyvinyl chloride (PVC) forms hydrochloric acid when it degrades, causing a chain reaction that rapidly proceeds to a complete loss of product strength. Organotins (OT), principally dibutyltin (DBT) and monobutyltin (MBT), are used as stabilizers in PVC pipe. Not all of the stabilizers used bind to the PVC polymer chains and, as a result, unbound stabilizers may migrate from polymer chains out of the pipe into transported waters. Limited occurrence data from residential surveys show levels of MBT and DBT in the parts per trillion (ppt) range. Organotins have been shown to have developmental neurotoxicological and immunotoxicological effects in animal studies. Because of its noted toxicity and potential for occurrence in drinking water, the U.S. Environmental Protection Agency (EPA) wants to characterize human exposures to OT via drinking water distribution systems and the risks of such an exposure. The goal of this research is to estimate OT concentrations in drinking water as a result of PVC/CPVC pipe leaching by integrating laboratory estimates of OT leaching rates and concentrations with estimates of average water distribution system parameters assuming minimal diurnal and intradistribution variability in concentrations unaffected by spikes in water quality or changes in treatment operational parameters. Metrics of human exposure via multiple-media/multiple-pathway probabilistic models are derived, addressing the utility of physiologically based pharmacokinetic models to address multi route extrapolation within these models.

Toxicology of Mono- and Di-alkyltin Chlorides

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Mono- and di-alkyltin chlorides are reactive compounds used in the production of stabilizers for polyvinyl chloride (PVC) plastics, primarily used for water distribution pipes. Health effects data were compiled or developed by the manufacturers for the EPA's HPV Challenge program for methyltin trichloride, (MMTC, CAS# 993-16-8), dimethyltin dichloride (DMTC, CAS# 753-73-1), butyltin trichloride (MBTC, CAS# 1118-46-3) and dibutyltin dichloride (DBTC, CAS# 683-18-1). These compounds are negative in the Ames test and negative to weakly positive in the mouse micronucleus test. In 90-day feeding or drinking water studies in rats, the nervous system is the primary target organ for methyltins. Microscopic changes were observed; NOAEL values were ~10 (MMTC) and 0.6 mg/kg (DMTC). Dietary administration of MMTC to rats for 2 weeks premating, then until gestation day (GD) 4 resulted in decreased numbers of pups and increased pup mortality. DMTC caused a dose-dependent reduction of maternal body weight (BW) gain of dams treated orally on GD 7-17. Pups demonstrated a reduction in mean BW and skeletal and visceral malformations. The NOAEL for both dams and pups was ~10 mg/kg for both MMTC and DMTC. 90-day dietary administration of MBTC caused increased liver weights, clinical chemistry changes and thymic atrophy. NOAELs were 525 mg/kg (90d, MBTC) and 0.4

mg/kg (28d, DBTC). MBTC demonstrated a reproductive & developmental NOAEL of 525 mg/kg. DBTC exposure (NOAEL) decreased numbers of pups delivered (2 mg/kg), increased pup mortality, and reduced dam BW gain and food intake (0.3 mg/kg).

Moser and Ehman reported that to assess developmental neurotoxicity using MMT, DMT and DBT, female Sprague-Dawley rats were exposed via drinking water through most of gestation and all of lactation; two studies included exposure prior to mating. Various neurobehavioral tests were used to assess neuromotor development and cognitive function from before weaning to adulthood. Two MMT studies, using concentrations up to 500 ppm, showed no effects on any measure of growth, development or cognitive function. The neuropathological evaluation revealed mild cortical vacuolation. Two studies of DMT also revealed few developmental effects; however, both studies demonstrated a reproducible effect on spatial learning (mid-concentration, 15 ppm, only), and mild cortical vacuolation was observed in one study. Brain weight was decreased in DBT-exposed offspring (25 ppm) at weaning. Assays of apoptosis revealed changes in the DMT and DBT-treated rats at several ages, indicating an altered progression of naturally-occurring cell death processes; however, these findings were not supported using another measure of apoptosis. Overall, these studies showed that: (1) MMT is the least biologically active organotin of the three tested, (2) both DMT and DBT produced some neurotoxicologically significant changes, but (3) the pattern of effects was quite different for these two compounds.

In immunotoxicity studies conducted by DeWitt, Copeland, and Luebke*,* body weight, immune organ weights, delayed-type hypersensitivity (DTH) responses, and natural killer (NK) cell activity were not affected in adults exposed to either 10 or 25 mg DBTC/L or 20 or 40 mg DMTC/L of drinking water for 28 days. Antibody responses in males exposed to DBTC as adults differed in two replicate experiments: IgG was elevated at the highest dose in one replicate whereas IgM was suppressed in the second. No changes were observed in DMTC-exposed adults. Immune organ weights, DTH responses and NK cell activity were not affected in offspring exposed pre- and post-natally via maternal dosing only (maternal, 10 or 25 mg DBTC/L of drinking water) or via the dam plus 10 direct doses by oral gavage from post-natal day 3-24 (maternal, 10 or 25 mg DBTC/L of drinking water; direct, 1.0 or 2.5 mg/kg BW/dose). BW gain of offspring in the maternal + direct 25 mg DBTC/L group was decreased relative to controls. IgM synthesis was suppressed only in maternal + direct female offspring. In contrast, IgG synthesis was elevated in maternal only male offspring. In adults and offspring, these effects on antibody production occurred at 25 mg DBTC/L of drinking water, a concentration several orders of magnitude higher than DBTC levels reported in drinking water. Effects on antibody synthesis only occurred at 25 mg DBTC/L, a concentration several orders of magnitude higher than levels reported in drinking water. The apparently contradictory effects on humoral immune function suggest that DBTC is unlikely to cause immunosuppression at levels demonstrated in drinking water. Furthermore, although the developing immune system is generally more sensitive than that of adults, our DBTC results suggest that at calculated levels of human exposure, developmental immunotoxicity is unlikely.

In summary, for methyltins consistent neuropathological findings were reported by several investigators, the nervous system effects indicate that DMTC is more potent than MMTC, and developmental neurotoxicity findings were not dose related. Although some reduction in thymus weight was reported for DMTC, no functional deficits were noted for this material. The NOAELs for reproductive effects, maternal toxicity and developmental effects were the same for MMTC. For DMTC, NOAELs for maternal toxicity and developmental effects were the same. For butyltins, the nervous system is not an adult target organ, and the developmental neurotoxicity findings reported for DBTC were different than those for the methyltins. Some morphology and

weight changes of the thymus, and changes in NK cell activity and immunoglobulin titers were noted for DBTC, but no delayed type hypersensitivity was demonstrated. Reproductive and developmental effects were noted for DBTC but not for MBTC. *(This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)*

Application of Mode of Action and Dose-Response Information in a Mixtures Risk Assessment

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Humans are exposed daily to environmental mixtures of chemicals and these mixtures can be simple, consisting of two to three compounds, or may be more complex containing several hundred related and/or unrelated constituents. Chemical constituents of a mixture can elicit similar, independent or interactive modes of action. Mode of action (MOA) for noncancer causing compounds, as defined by The United States Environmental Protection Agency (U.S. EPA), includes a sequence of events and processes, starting with interaction of an agent with a cell, proceeding through functional and/or morphological changes, resulting in toxicity of a tissue or organ system. The U.S. EPA utilizes toxic MOA data, when available, for risk assessment of chemical mixtures and employs the information in selection of an appropriate analysis model (e.g., dose addition, response addition, interaction indexes). Unless data are available to indicate an interaction of MOA among compounds in a mixture, the mixture risk assessment will be based on the assumption of additivity and a component-based approach taken. In that case, either dose addition (i.e., relative potency factor approach) or response addition will be selected, based upon data that can inform whether these compounds act via similar or independent modes of action, respectively. The success and acceptance of a mixtures risk assessment depends heavily on interpretation of available mixture study results and the description of MOA, as well as dose-response concordance or independence of toxic MOA, for mixture components.

SESSION 4B: NANOPARTICLES: TOXICOLOGY, HEALTH EFFECTS, HAZARD IDENTIFICATION

Risk Assessment and Medical Surveillance

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Amid the promise, rhetoric and uncertainty about nanotechnology, increasing numbers of workers are involved in research, development, production, and application of nanomaterials and products. Concerns about nanoparticle size and reactivity have prompted researchers and health authorities to investigate potential health effects from occupational exposures. The continuing need for efforts in hazard identification, risk assessment, characterization, communication and management is supported by findings from studies of air pollution, welders and diesel exposures, and animal exposures to ultrafine particles as well as by preliminary animal studies of engineered nanoparticles.

The most meaningful assessment of risks takes workplace preventive measures into account. The primary means of prevention are design measures and engineering controls, followed by administrative controls, training and work practices. Medical surveillance is a secondary means of prevention; it consists of either the statistical evaluation of groups of workers or the application of a test (medical screening) to individual asymptomatic workers. Established criteria need to be considered before implementing a medical screening program for workers exposed to nanomaterials and products.

Nanoparticles are Capable of Producing Reactive Oxygen Species, Causing Increased Cytotoxicity, and Altering Gene Expression

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Rationale: Nanoparticles are being developed for use in a number of fields ranging from computer to biological applications. As they continue to be identified for novel uses, caution must be used since their toxicological profile has yet to be determined. It is not known if having the nanomaterials in a cellular environment is capable of producing a direct toxic effect, or how the size, surface chemistry and/or composition could influence this response. The impact of uptake versus cellular contact also needs to be considered.

Methods: Nanoparticles have been investigated in a cell-free system for evaluation of their reactivity. Additionally, pulmonary cell lines were utilized for the analyses. They were exposed to nanoparticles under a variety of different conditions to examine the impact of uptake and particle type. Following incubation, cells were analyzed for reactive oxygen species (ROS) production and Lactate Dehydrogenase (LDH) release, as an index of cell death, and inflammatory cytokine expression, as a marker of changes in gene expression.

Results: Nanoparticles were capable of generating ROS in a cell-free system. The relative reactivity of particles was also demonstrated in the *in vitro* analyses. A dose dependent association of LDH release and cytokine expression was observed that was dependent on the chemistry of the material.

Conclusions: Nanoparticles are capable of producing ROS in both a cell-free and a cellular system. This production of ROS has the potential to damage lipids, proteins or DNA and may be the cause of increased cytotoxicity and changes in gene expression. *(Supported by NIEHS training grant T32 ES07026, NIH grant ES 01247, AFOSR grant FA9550-04-1-0430, EPA STAR PMCenter R-827354.)*

Challenges Associated with Health Risk Assessment of Nanomaterials: More Than Just Size

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Nanotechnology is a dynamic and enabling technology capable of producing a wide diversity of nano-scale (<100 nm) materials displaying unique electrical, catalytic, thermal, mechanical or imaging properties for a variety of applications. Nanomaterials may display unique toxicological properties and routes of exposure due to their novel physical chemical properties and applications. Although the existing publication database has provided information on particle dosimetry, fate and toxicity, initial nanoparticle health effect studies have shown some of them to display unique toxicities and mechanisms of injury. In addition, several international and national risk assessment reports have identified significant gaps in our knowledge regarding the health, ecological and environmental implications associated with manufactured nanomaterials and their applications. Research is critically needed in order to determine the health risk associated with manufactured nanomaterials, nanotoxicology. Preliminary toxicological studies have demonstrated manufactured nanoparticle toxicity to be extremely complex and multi-factorial, being regulated not only by size and shape but also by surface properties such as charge, area and reactivity. Linking adverse health effects to nanoparticle properties is critically needed in order to determine what metric best correlates with their toxicity. In addition, toxicological assessment of manufactured nanomaterials will require consideration of both local and systemic toxic responses due to their ability to be distributed beyond the initial site of deposition. Finally, a multi-disciplinary approach will be required in order to fully assess the health, ecological and environmental implications of manufactured nanomaterials and their applications. Research providing insight into the health and environmental implications of manufactured nanomaterials is critical to assess their potential risk and allow this enabling technology to develop in a responsible manner. *(This abstract does not reflect EPA policy.)*

Physical and Chemical Characterization of Nanoparticles

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Tremendous progress has been made during the past decade in the measurement of physical and chemical properties of nanoparticles. Particle size distributions down to 3 nm can now be measured routinely, and techniques have been developed to measure physical properties (e.g., density) and transport properties (e.g., dynamic shape factors). Such properties are important in assessing risk because they determine where particles will deposit in the respiratory system. A number of mass spectrometric techniques have also been developed to measure nanoparticle chemical composition. Two of these instruments are available commercially, while the others are laboratory prototypes. In this talk, current capabilities for measuring the physical and chemical properties of aerosols will be discussed.

Nanomaterial Exposure and Risk for Systemic Effects

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The most attractive features of nanomaterials including their small size, large surface area and reactivity might also be the main factors for their toxicity. In this regard, nanoparticles may induce not only higher damage at the penetration site but also can lead to unexpected distant responses as a result of their translocation and reactivity through the body. Our research efforts are currently directed to evaluate the cardiovascular effects, vascular inflammation and atherosclerosis, as well as the related molecular mechanisms associated with carbon nanotube (CNT) exposure using animal models. We demonstrated that pulmonary exposure to multiple doses of CNT induces severe lung toxicity and accelerates the progression of atherosclerosis in ApoE-/- mice. This response is accompanied by oxidative modification in the vascular wall. The atherogenic effects might be a result of a low-grade systemic inflammatory response related to the lung toxicity and/or translocation of nanotubes into the systemic circulation. The accumulation of toxicological data on engineered nanomaterials will allow for development of adequate risk assessment and regulations.

SESSION 4C: NEW APPROACHES FOR ASSESSING THE HEALTH EFFECTS FROM EXPOSURES TO CHEMICAL MIXTURES

Non-additive Interactions of an Organophosphorus Pesticide Mixture in Adult and Preweanling Rats

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Critical features of risk assessment include the evaluation of risk following exposure to pesticide mixtures as well as the potential for increased sensitivity of the young. The U.S. EPA is required to regulate pesticides acting via a common mechanism of action as a group, e.g., cumulative risk assessment. The first common mechanism group identified was the organophosphorus (OP) pesticides which inhibit acetylcholinesterase. The current default assumption is dose-additivity for mixtures with a common mode of action. There are, however, literature reports on binary OP mixtures showing non-additivity in about half of the pairs tested, as well as synergy produced by malathion in combination with other certain OPs. This research tested for interaction(s) using a mixture of five OPs (chlorpyrifos, diazinon, dimethoate, acephate, and malathion) as well as four OPs (without malathion) in both adult and preweanling (17-day-old) rats using a fixed-ratio ray design. The pesticide ratio was based on the relative dietary exposure estimates. Neurochemical (blood, brain cholinesterase activity) and behavioral (motor activity, gait score, tail-pinch response score) endpoints were assessed following acute oral exposure. To determine age-related differential responses, we conducted the study in both adult and preweanling (17-day-old) rats. Single chemical dose-response data were used to construct a theoretical additivity model for each mixture. Mathematical curves fit to the empirical data were statistically compared to the predicted curves to determine deviations from dose-additivity. In both adult and preweanling rats, the analyses revealed significant greater-than-additive (synergistic) responses for blood and brain ChE inhibition, motor activity, gait alterations and tail-pinch response (pups only). Most often, the deviations occurred at the low end of the curves, and effective doses (ED_{20}, ED_{50}) of the mixture were 2- to 3-times less than predicted under additivity. Comparing the full and reduced rays showed that malathion interacts with the other OPs for some endpoints; however, the deviation from additivity cannot fully be attributed to the malathion in the mixture. The results from these studies indicate that under certain experimental parameters, deviations from additivity are observed with these common mode of action pesticides. Correlating these findings with tissue levels of pesticide and kinetic models may allow mechanistic explanations for these deviations, and better predictions for their occurrence. *(This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)*

The Effects of Jet Fuel on the Airway and Immune Function

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Each year more than a million military and civilian personnel are occupationally exposed to jet fuel in the United States. Published studies have reported both short-term and persisting health effects, including pulmonary and immune responses following acute and chronic jet fuel exposure. The purpose of this study was to determine the acute effects of Jet-A on the upper and lower airways as well as the immune system. Female Sprague-Dawley rats (n=24/group) were exposed to a mixed vapor/aerosol Jet-A atmosphere (500, 1000 or 2000 mg/m^3) or filtered

air (control) for 4 hours/day, 7 days/week for 14 consecutive days in whole body inhalation chambers (690 liters). Twenty-four hours, 7 and 14 days after the last inhalation exposure, the animals were sacrificed; the lungs and nasal cavities were lavaged with saline and markers of inflammation and injury were measured in the lavage supernatants. In the lungs, the only significant changes compared to controls were an increase in total protein (µg/ml) in the 1000 mg/m^3 exposed rats on day 14 and an increase in lactate dehydrogenase (LDH; ng/ml) in the 500 mg/m^3 group on day 7. In the upper airway, total protein and LDH were both significantly elevated in the 2000 mg/m^3 exposed animals compared to controls. Spleens were also harvested for spleenic phenotyping, but no significant alterations in lymphocyte and myloid/neutrophil populations were observed in the jet fuel exposed animals compared to controls. These results suggest that acute exposure to high concentrations of Jet-A may have minimal pulmonary effects.

Low-Dose Mixture Effects of PCB126 and Perchlorate on the Male Rat Hypothalamic-Pituitary-Thyroid (HPT) Axis

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Perchlorate (ClO_4^-) and 3,3',4,4',5-pentachlorobiphenyl (PCB126) are environmental contaminants known to disturb thyroid hormone homeostasis by well defined modes of action that lead to hypothyroidism in the rat. PCB126 increases phase II conjugation of T4 (T4-glucuronide) by inducing hepatic uridine diphosphate glucuronyl transferases (UDPGTs), leading to increased excretion of T4-glucuronide in the bile and feces. The perchlorate anion competitively inhibits iodide uptake into the thyroid at the Na^+/I^- symporter (NIS), leading to iodide insufficiency in the thyroid and reduced thyroid hormone synthesis (Yu *et al.*, 2002).

Two experiments were conducted to investigate the HPT axis effects of ClO_4^- on rats pretreated with PCB126. For the first experiment, adult male Sprague-Dawley rats were administered a single oral bolus dose of 0, 7.5, or 75 µg PCB126/kg-bw in corn oil on Day 0. On Day 9, ClO_4^- was administered in drinking water (DW) to obtain target doses of 0, 0.01, 0.10, or 1.0 mg/kg-bw per day for 14 days. Rats were euthanized on Day 22. Data were analyzed by ANOVA followed by Tukey's multiple comparison test at $p<0.05$ using SAS v8.2 (SAS Institute, Cary, NC). Hepatic EROD activity, a marker for CYP1A1 activity, was significantly induced for all animals treated with PCB126. Perchlorate did not alter hepatic EROD activity. A PCB126 dose-dependent increase in hepatic T4-G formation was observed, with statistical significance found only for the highest dose group. TSH serum levels were significantly increased above controls in both the 7.5 and 75 µg PCB126/kg-bw dose groups. All rats dosed with 75 µg PCB126/kg-bw had significantly reduced total and free T4 serum concentrations when compared to controls, suggesting that in the 7.5 µg PCB126/kg dose group, TSH-induced upregulation of the thyroid compensated for the thyroid toxic effects of PCB126. Serum total and free T4 concentrations were similar to controls for animals treated with ClO_4^- alone; however, ClO_4^- resulted in a dose-dependent increase in TSH, with statistical significance observed at the highest dose group. Thus, it appears that TSH induced stimulation of the thyroid was sufficient to compensate for the initial reduced thyroidal iodide uptake and maintain adequate hormone synthesis. When ClO_4^- was administered to rats pretreated with PCB126,

the effects on most measured HPT axis indices were less than additive to additive compared to the expected values determined by addition of individual chemical responses. However, further studies were required to examine the interactions of ClO_4^- and low doses of PCB 126, which do not increase TSH serum concentrations, hepatic EROD activity, or hepatic T4-G formation.

The second experiment was designed to examine the effects of lower doses of PCB126 on the HPT axis at earlier time points than collected in the first experiment. In this experiment, adult male Sprague-Dawley rats were administered a single oral bolus dose of 0, 0.075, 0.75, or 7.5 µg PCB126/kg-bw in corn oil on Day 0. On Day 1, ClO_4^- was administered via DW (0 or 0.01 mg ClO_4^-/kg-bw per day) and rats were sacrificed and tissues collected on Days 2 and 5. Dose and time dependent induction of hepatic EROD activity was observed over the 5 day period for all but the lowest dose of PCB126. Previous work with higher doses of PCB126 showed that the peak rate of hepatic T4-glucuronide production was 5 days post PCB126 dose (Fisher *et al.*, 2006); however, this study determined peak production as early as 2 days post PCB126 with return to near control rates by 5 days (in agreement with the 7.5 µg PCB126/kg-bw from Fisher *et al.*, 2006). As expected there was no significant effect of ClO_4^- on T4-G formation. Surprisingly, rats that received ClO_4^- in DW for 4 days had modestly elevated mean serum total T4 concentrations, though not statistically significant; however, a less than additive response was observed when pretreated with PCB126. Thyroidal iodide (^{127}I) stores were not altered by these chemicals and ranged from 10-15 µg (both lobes) for control and treated groups. Overall these study conditions demonstrated that animals pretreated with PCB126 followed by exposure to ClO_4^- exhibited responses ranging from less than additive to additive for several HPT axis indices. The less than additive effects, based on mean responses, may have occurred because PCB126 caused an initial stimulation of the thyroid gland by TSH resulting in increased NIS expression, such that the blocking of iodide uptake by ClO_4^- was not as potent as in euthyroid animals.

A biologically-based pharmacodynamic model of the HPT axis is under development in our laboratory. This model, linked with physiologically-based pharmacokinetic models for ClO_4^- and PCB 126, will help interpret the nonlinear HPT axis disturbances that apparently involve both stimulation and inhibition. *(This abstract does not reflect U.S. EPA policy. Funding was provided by ATSDR #U61/ATU472105-01, U.S. EPA, and NSF fellowship (DGE0229577).)*

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Simulation Modeling of Pharmacological Intervention for Chemical Warfare Agent Exposure (CWA) Affects

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The primary mode of toxicity for chemical warfare agents of the organophosphorus type is irreversible inhibition of acetylcholinesterase (AChE), resulting in excessive amounts of acetylcholine (ACh) at cholinergic synapses. Current medical therapies across the world employ the same basic approach to counteract this threat, using the same three strategies of treatment: an anticholinergic drug to address acute cholinergic stimulus, a reactivating oxime to free inhibited AChE, and a specialized anticonvulsant to treat or prevent seizures and resultant neuronal damage. Verification of this treatment schedule and any newer treatment methods is dependent on extrapolation of animal results to predict human efficacy. An approach that can make this process more quantitative would employ pharmacokinetic and pharmacodynamic models, which simultaneously tract the concentration of the CWAs and therapy drugs at the different target tissue sites, thus allowing a significantly improved extrapolation from a more extensive animal data base, with actual use of CWAs, to human exposure and treatment scenarios. Current attempts to develop this approach from the open literature will be discussed and the use of physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) models as an approach will be presented.

PBPK Model for Selected Aromates in a JP-8 Vapor Exposure System

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JP-8 is a kerosene based fuel and includes alkanes (C_7 to C_{19}), aromates (toluene, xylenes, trimethylbenzenes, naphthalenes). With the ultimate goal of this research being to develop a total hydrocarbon PBPK model for JP-8 using marker compounds for several of the chemical classes found in the JP-8 (the aromatic fraction is just one class), we have developed a PBPK model for several aromates including m-xylene, 1,2,4-trimethylbenzene, ethylbenzene, and toluene to begin to assess possible interactions of JP-8 vapor on individual component kinetics.

JP-8 inhalation studies with rats were carried out in a 31 L leach chamber exposure system. Rats (8-12 per run) were exposed to JP-8 vapor generated with a diffusion bubbler and diluted with room air to generate a total flow of 8 L/min. Exposure of rats to JP-8 vapor was carried out for 4 h at three concentrations (384, 1085, or 2680 mg/m^3). Individual component concentrations in the chamber atmosphere were determined from charcoal tubes (50/100) collected during the run and analyzed by GC-MS after desorption with chloroform.

Tissue (blood, brain, liver, and fat) were harvested at the end of exposure for all three concentrations. For the 2680 mg/m^3 exposure, additional tissues were harvested during exposure (2 h) and 0.5 h post exposure. Fat was collected up to 44 h post exposure (2680 mg/m^3 exposure). Tissues were analyzed by a novel headspace SPME/GC-MS technique that was developed in our laboratory. Samples were prepared by placing a weighed quantity of chopped tissue or blood into a 10 ml headspace vial and adding 3 mL of 33% salt water and 150 ng of internal standard (d_{26}-dodecane in 1 µl of chloroform). After heating the sample for 45 min at 65^{o}C the SPME fiber (100 µm PDMS) was exposed to the headspace of the vial in order to adsorb the analytes onto the fiber for introduction to the GC-MS (Varian 3800 GC/Saturn 2200 ion trap MS).

Concentrations in blood at the end of exposure ranged from 444 ng/ml (m-xylene) to 20 ng/ml (tridecane) for the 2680 mg/m^3 exposure. In the liver, hydrocarbon concentrations ranged from 543 ng/g (m-xylene) to 6.0 ng/g (tridecane). The clearance of these hydrocarbons from blood, liver, and brain was rapid with concentrations of all components monitored decreasing from 30 – 70% by 0.5 h post-exposure.

A five compartment model (blood, liver, slowly perfused, richly perfused, brain, and fat) was developed for each of the four aromates. Fat has a large influence on blood and liver predictions for lipophilic substances. Diffusion limited equations were added to the description of fat and brain compartments to account for the slow uptake and clearance of the four aromates from these tissues. It was necessary to refit metabolic constants that had previously been determined using flow limited for fat (Haddad *et al.*, 1999). In the case of 1,2,4-trimethylbenzene this was the first effort to simulate rat kinetics, and metabolic constants for this compound were fit to predict previously published blood data (Swiercz *et al.*, 2003).

With the addition of diffusion limitation for the fat and brain, the model predictions of measured concentrations for the four aromates were improved greatly for the data collected at the end of exposure to JP-8 vapor as well as the previously published blood data. Overall, this pharmacokinetic analysis suggests that metabolic interactions are minimal for m-xylene, 1,2,4-trimethylbenzene, ethylbenzene, and toluene in the presence of JP-8 vapor.

References:

Haddad *et al.* 1999. Toxicol. Appl. Pharmacol. 161:249-257.
Swiercz *et al.* 2003. Int. J. Occup. Med. Environ. Health 16(1):61-66.

Physiologically Based Pharmacokinetic Modeling of the Neurotoxicity of Mixtures

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Cumulative risk for AChE-inhibiting pesticides, including organophosphates and N-methyl carbamates, is calculated from the degree of AChE inhibition predicted for the mixture, with AChE inhibition being used as a surrogate for frankly neurotoxic effects. The default approach for cumulative risk assessment ignores the potential for interactions between different pesticides. Two types of interactions are likely: competition for metabolism and interaction at AChE. Assessing the nature and extent of these interactions on the basis of administered dose can be misleading due to the complications introduced by pharmacokinetic differences between the chemicals. PBPK modeling for AChE-inhibiting pesticides could in principal be used to predict cumulative risk on the basis of interactions at the target tissue, while taking into account any metabolic interactions. Although fully developed examples of cumulative assessments based on PBPK modeling do not yet exist, prototypical simulations using PBPK models for chlorpyrifos and carbaryl will be presented. The individual PBPK models were linked together by describing interactions at sites of metabolism and at AChE. The resulting “mixture model” can describe the overall degree of AChE inhibition for the mixture as a function of (1) the individual pharmacokinetic behavior of each chemical and (2) the chemical-specific kinetics of AChE inhibition. The extension of this approach to mixtures that include pyrethroids will also be discussed. Since pyrethroids produce neurotoxicity through a mode of action not involving AChE, there is a potential for interactions between pyrethroids and AChE inhibitors that are different from additive or competitive.

POSTER SESSION

Opinions of Experts: Peer Review, Peer Consultation, and Expert Elicitation

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Toxicology Excellence for Risk Assessment

While peer review has been used for many years to judge the scientific quality of work products, expert elicitation and peer consultation have recently been introduced to improve the quality of risk assessment documents under development. There is some confusion regarding these concepts. These three methods of soliciting expert opinion have common features, including highly qualified scientists with appropriate expertise to evaluate the product and clear instructions to these scientists. This poster will compare and contrast peer review, peer consultation, and expert elicitation as they are used in the field of risk assessment. The similarities and differences among these approaches, in their goals and objectives, procedures used, and results, will be described. The value of each of these approaches in various situations will be discussed. *(This work has been funded in part by the U.S. EPA (cooperative agreement X-82916801); however, the views expressed in this poster are those of the authors and do not represent the views of the funding agency.)*

Gender Based Preliminary Pediatric Studies for Serum Amyloid A (SAA) and C-Reactive Protein (CRP)

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Serum Amyloid A (SAA) and C-Reactive protein (CRP) are acute phase reactants that increase in a number of inflammatory, infectious and neoplastic disorders. CRP concentration is commonly used as a non-specific test to assist in the diagnosis of infection. Data supporting the efficacy of SAA as an early indicator of pediatric infection are very limited and conflicting. Our laboratory has recently reported that over 1/3 of healthy infants born vaginally had SAA levels >10 µg/mL, a level previously considered to be the upper limit of normal range for adult populations. Despite frequent clinical use, there is little data defining normal pediatric CRP levels and comparing CRP with SAA concentrations. Additionally, the laboratory methodology to measure SAA has been time consuming and cumbersome. The objectives of this study were threefold: (1) to determine the SAA and CRP levels in non-infected male and female children (2) to compare these levels to children with acute bacterial and viral infections (3) to develop and test an automated method for SAA quantative analysis.

SAA quantitative analysis was done using the PersonalLAB™ (AdaltisRobotics, Italy) open system, using an in-house designed automated immunoassay. CRP was measured with Beckman Coulter system LX20PRO using a highly sensitive near Infrared Particle Aminoassay Rate method. Statistical analysis of the SAA coefficient of variation (CV%) showed that the manual method was not markedly different from the automated method regardless of whether the old (z = -0.082; p = 0.935) or the new (z = -1.892; p = 0.058) lots were used. There was no significant difference in the optical density (a raw data measure of SAA concentration) using the

manual vs. the automated method (p = 0.40). The seamless transition from manual to automated SAA ELISA proved to maximize throughput.

Over a six-month period, residual serum from clinically indicated laboratory tests was obtained from children whose ages ranged from seven months to 18 years, and was stored at -80ºC until analyzed. Medical records were reviewed and patients with underlying chronic diseases such as asthma, acne, cancer, inflammatory bowel disease, or receiving anti-inflammatory drugs, etc. were not studied, resulting in a total of 188 patient serum specimens eligible for the study. Non-parametric Kruskal-Wallis and Wilcoxon tests were used to compare the group study categories (no infection, bacterial or viral) and to examine any sex differences within each of the infection categories for the CRP and SAA measurements

We found significant gender differences in SAA values within the non-infected group (males median = 4.81, females median = 9.77 µg/mL; $^{*}p<0.05$). We therefore examined SAA differences between the infection groups separately for males and females. For males, the bacterial infection group was significantly different from the non- infected group (median = 151.08 vs. 4.81; $p < 0.0001$). Also, males with viral infection were significantly different from the non-infected group (median 27.57 vs. 4.81; $p = 0.005$). Unexpectedly, females had no significant differences in SAA concentrations between the three groups. In contrast, there were no gender differences in the CRP concentrations. CRP concentrations were significantly higher in both the bacterial group as compared to the no infection group (median = 0.42, vs. 0.09; $p<0.001$) and the viral infection group compared to the no infection group (median = 0.34 vs. 0.09; $p = 0.004$).

While our study confirmed the value of CRP as an adjunctive test in the diagnosis of infection, the lack of a significant rise in SAA concentration in acutely infected females was unexpected. Further prospective studies are needed to verify these preliminary findings, including pre- and post-puberty data analysis.

Development of Quantitative Structure Activity Relationships to Predict Toxicity for a Variety of Human and Ecological Endpoints

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The number of chemicals released into the environment has significantly increased over the past few years, leading to increased risk of human exposure through inhalation, ingestion, or dermal uptake. In addition, the risk also increases with increasing toxicity of the chemical. A number of ranking schemes that are based on exposure and toxicity have been developed to aid in the prioritization of research funds for identifying chemicals of regulatory concern. However, there are significant gaps in the availability of experimental toxicity data for most health endpoints. Predictive toxicological approaches such as Quantitative Structure-Activity Relationships (QSARs) provide a means to estimate the toxicities for chemicals that lack experimental data. For the purposes of this study, QSARs are mathematical equations that relate the toxicity of a chemical to its physicochemical properties calculated using Kier and Hall type indices (2-dimensional descriptors) and computational quantum chemistry (3-dimensional descriptors).

The objective of this study is to construct a software tool to predict the oral rat lethal doses (LD_{50}s) of a wide variety of chemicals. The software tool will be coded in Java and accessible through the Web. The user will simply input a chemical to be evaluated by drawing it in a 2-D chemical sketcher window, entering a SMILES string, or entering a CAS number. To build the tool, a database containing LD_{50}s of approximately 3000 chemicals was gathered from the literature. Algorithms for calculating 2-dimensional descriptors such as connectivity, shape, E-State, and other information indices are being developed in Java, and the descriptors calculated by the program are validated against those calculated by the descriptor generator programs ADAPT, Dragon, MDL QSAR and Molconn-Z. The 3-dimensional descriptors such as partial charges and surface areas are calculated using freely available platform-specific programs such as MOPAC and TINKER. Algorithms for generating these descriptors are being developed in Java and validated against AMPAC, MOPAC, NWChem and TINKER. C/C++ and Java based algorithms are also being developed that group the chemicals in the database into clusters of chemicals with similar physicochemical properties (based on Ward's method), and generate the QSAR equation to predict the LD_{50} of a chemical from its physicochemical properties for each cluster using parallelized genetic algorithms. The chemicals in each cluster and their predicted LD_{50}s will be validated against those generated by MDL QSAR and SAS.

Development of Structure Activity Relationship Models to Predict the Rat Carcinogenicity of Chemicals

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Long-term animal bioassays for carcinogenicity are regularly used to determine whether chemical agents may be capable of inducing cancer in humans. In addition to time and cost, two important disadvantages of current bioassays are that testing covers a substantial portion of the lifespan of the test species, and that high doses are usually used in the testing process. These disadvantages may be circumvented using Quantitative Structure-Activity Relationships (QSARs), which have the added advantage of minimizing the use of animals for testing purposes. There are a limited number of QSAR models available in the literature that predict the carcinogenicity of chemicals. Most of these models relate the potency to measures of carcinogenicity such as mutagenicity, lethal dose (LD_{50}) or the maximum tolerated dose (MTD). For example, Krewski *et al.* (1989) demonstrated a good correlation ($r = 0.952$, $N = 191$) between the tumor dose that causes cancer in 50% of an exposed population (TD_{50}, a measure of carcinogenic potency) and the MTD.

The objective of this study was to develop a QSAR model to predict the carcinogenic potency of a wide variety of chemicals. Since a majority of the QSAR models available in the literature today relate the TD_{50}s to MTD, this study aimed to duplicate the results of Krewski *et al.* (1989) using data published TD_{50}s in the Carcinogenic Potency Data Base (CPDB) (Gold, 2005). The MTDs, LD_{50}s and LOAELs of 467 chemicals from the CPDB were estimated using TOPKAT, a QSAR software for toxicity prediction. The correlation between TD_{50} and MTD for the 467 CPDB chemicals ($N = 467$, $r = 0.538$) was poor compared to the result from the study by Krewski *et al.* (1989). Since the MTD model in TOPKAT was not useful for predicting the TD_{50}, this study developed QSAR models using both TD_{50} and oral slope factor (OSF; 95% upper bound on the dose-response slope) as measures of carcinogenic potency. The TD_{50}s were obtained from the CPDB while OSFs were obtained from U.S. EPA's IRIS database. The lowest energy

conformers of the chemicals considered in this study were obtained using CAChe software. Four descriptor-generating programs were used to generate the descriptors for the QSAR model: Dragon, Molconn-Z, CAChe and AMPAC/CODESSA. Results of this research indicate that sex, species, and mechanism-specific QSARs have a greater predictive ability than generic QSARs that considered all chemicals irrespective of sex, species or mechanism of action. In addition, while the QSARs developed in this study may predict the carcinogenic potency of a wide variety of chemicals, they may not be able to differentiate between carcinogens and non-carcinogens. In other words, they predict a TD_{50} for all chemicals irrespective of whether the chemical is a carcinogen or not. Hence, separate discriminative QSAR models may need to be developed to differentiate carcinogens from non-carcinogens.

Human Health Risk Assessment Tools: Supporting the Risk Assessment Process at the National Homeland Security Research Center

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EPA's National Homeland Security Research Center (NHSRC), Threat and Consequence Assessment Division (TCAD), evaluates the human health risks associated with the release of contaminants into the environment. To support this evaluation, TCAD develops risk assessment methods and tools and risk communication techniques that will be of practical assistance to government agencies and first responders dealing with threats from chemical, biological, and radiological contamination. Two tools being developed to assist first responders, risk assessors, and decision-makers are the Data Dictionary (DD) and the Emergency Consequence Assessment Tool (ECAT).

The DD includes a compilation of primary and secondary data on toxicity, infectivity, dose-response, and health effects for NHSRC's priority list of chemical, biological, and radiological agents. In addition to providing pertinent information to risk assessors and managers, the DD will support the ECAT by providing toxicity information and benchmarks, it will support the development of Provisional Advisory Levels for human exposure and will be used by the Water Infrastructure Protection Division's (WIPD) Threat Ensemble Vulnerability Assessment (TEVA) modeling tool to fill in toxicity data gaps.

The ECAT is an interactive web-based software tool designed to provide a wide range of emergency response officials with accurate information in a rapid manner during a major environmental crisis caused by a terrorist attack or natural disaster. ECAT has been designed to include many critical features that will assist the needs of a wide range of emergency response officials including first responders, health advisors, and senior decision-makers. ECAT will be used as both a training tool and as an emergency response tool and is innovative in three ways. First, ECAT provides a holistic approach by integrating critical information across many scientific disciplines spanning the entire risk assessment and risk management paradigm. Second, ECAT provides instant access to key information and allows its users to conduct rapid analyses of complex data. Third, ECAT is versatile and can be applied by a wide range of users including first responders, health advisors, and senior decision-makers.

Both these tools will undergo rigorous internal and external peer review to ensure scientific credibility and technical quality. In combination, these tools offer a powerful means to quickly and efficiently gather pertinent information on the health risks posed by agents that are of

primary concern to the Nation's security. These tools will play an important role in understanding human health risks and will provide the means for risk assessors and managers within and outside the Agency to make informed decisions that can be utilized to conduct innovative human health risk assessments.

In Vitro Toxicology of Aluminum Nanoparticles in Rat Lung Macrophages

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Nanomaterials, which are by definition in the 1-100 nanometer range, have numerous possible benefits to society, but currently there is a lack of data that characterize these materials effects on human health and environment. In general nanomaterials are of interest to the Air Force because of their applications in electronics, sensors, munitions and energetic/reactive systems. Nanoparticles such as aluminum have been considered for enhancing propulsion in solid rocket fuel. To date, only a few studies have looked at the toxicological effects of direct exposure to nanoparticles, none with aluminum. It is important to increase the understanding of the nanomaterial exposure health impact before these materials are throughout diverse levels of occupations or fully used in large capacities within industry and the military. The purpose of this research is to observe and characterize the *in vitro* cellular effects of rat lung macrophages to exposure to aluminum oxide nanoparticles (30 and 40 nm average size) compared to pure aluminum nanoparticles (50, 80, and 120 nm). This study concentrates on cell viability, mitochondrial function, phagocytotic ability, cytokine response, and cell morphology. Preliminary viability results show minimal toxicological effects on these macrophages exposed as high as 500 µg/ml for 24 hours with aluminum oxide particles. However, there was significant toxicity that occurred at 96 and 144 hours post exposure indicating a delayed toxicity. Pure aluminum particles indicate sight to moderate toxicity after 24 hours exposure at 100 and 250 µg/ml. Preliminary results indicate that the phagocytic ability of these cells is hindered by the pure aluminum nanoparticles, but not by the aluminum oxide nanoparticles. A series of cytokine and nitric oxide assays are being performed to characterize possible enhanced immune cascade variations from normal and inflammatory responses.

Application of the U.S. EPA's Revised Guidelines for Carcinogen Risk Assessment and Mode of Action Framework: An Example with Aldrin/Dieldrin-Induced Mouse Liver Tumors

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The cyclodiene organochlorine pesticides aldrin and dieldrin are among a number of non-genotoxic mouse liver carcinogens currently classified as B2, "probable human carcinogens, based on the U.S. Environmental Protection Agency's (U.S. EPA's) 1986 *Guidelines for Carcinogen Risk Assessment.* These original guidelines provided no specific guidance for consideration of mechanistic information, but the final revised version, issued in March 2005, recognizes key advances in understanding of the molecular events underlying cancer induction processes, and their implications for evaluation and regulation of potential human health risk. Among the most significant changes to the guidelines are incorporation of a framework to

determine the human relevance of animal tumors as part of the hazard identification phase of the risk assessment process. This framework provides a scientific basis for making weight-of-evidence judgments about the relevance of animal tumors for human health based on understanding the biological events leading to an animal tumor response. Under this framework, if the weight of evidence is sufficient to establish a mode of action (MOA) in animals, and if the human relevance of the MOA can be reasonably excluded on the basis of qualitative differences in key events or quantitative differences in kinetic or dynamic factors between experimental animals and humans, then the MOA is deemed not relevant to humans. In the case of aldrin/dieldrin, the weight of experimental evidence indicates that these compounds act as accelerators of background liver carcinogenesis in mice through an epigenetic MOA similar to that proposed for the extensively studied drug phenobarbital. The key events resulting in the potential formation of mouse liver carcinogenesis are not anticipated in humans. The result is that the application of the revised U.S. EPA carcinogen risk assessment guidance for aldrin and dieldrin supports the conclusion that the most appropriate cancer risk descriptor for aldrin/dieldrin is "not likely to be carcinogenic to humans."

Interaction of Well-dispersed Nanodiamond Particles with Neuroblastoma Cells

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Owing to its low chemical reactivity and unique physical properties, nanodiamond could be useful in a variety of biological applications, including carriers for drugs, genes, proteins, or vaccinations; novel imaging techniques; tissue scaffold design; and many other emerging technologies. In the past, it has been difficult to separate individual nanodiamond particles. A new stirred-media milling technique can now be used to disperse individual nanodiamond particles of sizes ranging from 2-10 nm. The individual nanodiamond particles can then be rendered soluble in aqueous media by chemical functionalization with either acids or bases. Here, we present a study of the biocompatibility of raw and functionalized nanodiamonds in neuroblastoma cells for 24 hours with concentrations ranging from 5-100 µg/ml. These well-dispersed nanodiamond particles were found to agglomerate at cell borders and along their processes as well as becoming internalized by the cells after 24 hours, while remaining non-toxic. We further found that the cells can grow on nanodiamond-coated glass coverslips. These results suggest that nanodiamonds may be ideal for biocompatible implants or devices.

Integration of the Available Genomic Data for Inorganic Arsenic Species to Support the Development of a Nonlinear Cancer Dose-Response Modeling Approach

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Inorganic arsenic acts as both a carcinogen and a cancer therapeutic in humans, yet an animal model for the carcinogenic effects of arsenic remains elusive. This curious behavior has prompted the collection of extensive data on the genomic effects of arsenic compounds. A

mode of action based on these genomic interactions could potentially support an alternative to the current linear low dose risk assessment, but the dose-responses for these interactions have not been well characterized. In this effort, a comprehensive literature search was conducted that identified more than 400 studies containing quantitative information on the response of genes or proteins following exposure to arsenic compounds. This information was organized by arsenic compound, dose/concentration, species, and tissue/cell type, to characterize the dose-response for the interactions of arsenic with cell signaling pathways. The results of our analysis support a multi-pathway, dose-dependent mode of action for the carcinogenicity of arsenic. At sub-micromolar concentrations, arsenic treatment is associated with adaptive responses, including alterations in signal pathways associated with oxidative stress and proliferative signaling. At higher concentrations, arsenic treatment is associated with inhibition of DNA repair and alterations in signal pathways associated with cell cycle delay and apoptosis. The inferences derived from this experimental data have been used to design a genomic dose-response study that compares *in vivo* and *in vitro* genomic responses in the mouse bladder with *in vitro* responses in human bladder cells. This quantitative genomic data will be used in a biologically based dose-response modeling approach to support a nonlinear risk assessment for arsenic.

Size-dependent Toxicity of SiO_2 in Mouse Keratinocytes

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Nanotechnology is an emerging area in which many findings have been reported on distinctive properties of nanomaterials. Nanoparticles, which range in size from 1-100 nanometers, have been used to create unique devices at the nanoscale level possessing novel physical and chemical functional properties. Although manufactured nanomaterials are currently widely used in modern technology, there is a serious lack of information concerning the human health and environmental implications of these materials. In view of their possible effect on human health our main focus was to investigate toxicity resulting from nanoparticles exposure. The objective of this study was to examine toxic effects of SiO_2 by investigating cytotoxicity of silicon dioxide in mouse keratinocytes (HEL-30). Cells were exposed to different sizes (35, 51, 110 and 420 nm) of homogeneously suspended SiO_2 at various concentrations (0, 10, 50, 100 and 200 µg/mL) for 24 h. Results of LDH leakage in the media were dose- and size- dependent. Exposure of the 35 and 51 nm at 200 µg/mL at 24 h resulted in 70% and 40% of LDH leakage, respectively. However, no changes observed in both 110 and 420 nm in any concentrations. MTT assays were carried out to study cell viability based on the mitochondrial function. Size- and dose-dependent MTT reductions were observed with similar LDH leakage. Small sizes of silica such as 35 and 51 nm at high concentrations (200 µg/mL) produced significant toxicity when compared to large size (110, 420) particles. Reactive oxygen species (ROS) generation was observed to investigate if oxidative stress was induced by silica particles. No significant increase in ROS was observed in cells exposed to any sizes of particles. This shows that the toxicity of silica particles does not seem to be mediated by oxidative stress. Further investigations are underway to investigate if redox potential of cells (GSH/GSSG ratio) and mitochondria membrane potentials play a role in the mechanism of silicon dioxide toxicity.

Development and Evaluation of a GC Test Method to Quantify Urinary (2-Methoxyethoxy)acetic Acid using t-Butyldimethylsilane Derivatization

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An accurate and precise test procedure was developed to detect and quantify the level of (2-methoxyethoxy)acetic acid (MEAA) in human urine. MEAA is a metabolite and biomarker for exposure to 2-(2-methoxyethoxy)ethanol, a glycol ether with widespread use in various industrial applications and the specific use as an anti-icing agent in the military jet fuel formulation JP-8. Human dermal exposure to 2-(2-methoxyethoxy)ethanol is of concern because of the general toxicity of glycol ethers and the toxicity of this compound. Urine collection and testing would offer a noninvasive means of accessing exposure and could be used for toxicological risk assessment of exposed workers. Urine sample preparation consisted of liquid-liquid extraction (LLE) with ethyl acetate followed by silylation of the extracted MEAA with N-methyl-N-[tert-butyl-diemethylsilyl]-trifluoroacetamide (MTBSFA). Quantification was achieved using a gas chromatograph (GC) equipped with a mass selective detector (MSD) using a dimethylpolysiloxane (HP-1) capillary column. Deuterated 2-butoxyacetic acid (*d*-BAA) was used as a procedural internal standard for this test. Demonstrated accuracy and precision for this procedure's recovery study was good; recovery varied between 94 and 99% with relative standard deviations (RSD) of 7.3% or less using fortified urine samples. The limit of detection (LOD) for this test procedure was approximately 0.01 µg/mL MEAA in urine. This method demonstrated improved precision and a lower limit of detection over previously developed analytical methods. (*Disclaimers: Mention of company names and/or products does not constitute endorsement by the Centers for Disease Control and Prevention (CDC). The findings and conclusions in this abstract have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent any agency determination or policy*.)

The Search for Novel Protein Biomarkers of Exposure in Human Urine Using SELDI-TOF Mass Spectrometry

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This study is designed to identify molecular biomarkers in human urine that may be used to assess acrylamide exposure or early disease onset. Acrylamide (CAS 79-06-1), a widely used industrial chemical, which is also formed in thermally processed food, can produce peripheral neurotoxicity and reproductive effects. Acrylamide has been classified as a probable human carcinogen. The development of novel proteomic-based biomarkers to evaluate dermal exposure would be of value in assessing potential toxicity in workers. Changes in the levels of potential protein biomarkers in serum and urine have been reported for various toxic agents, and the bioactivation of acrylamide to glycidamide (5694-00-8) suggests that urinary protein levels would be affected. In the current study the utility of four types of Ciphergen ProteinChips were evaluated by comparing protein profiles in urine samples from control and industrial exposure samples using Surface Enhanced Laser Desorption Ionization Time of Flight (SELDI-TOF) mass spectrometry. Urinary proteins were processed on NP20, CM10, H50, and IMAC30 chips having normal phase, cationic exchange, hydrophobic, and ionic surface chemistries, respectively. The number of peaks and mean peak intensity varied significantly with IMAC30 > CM10 > NP20 > H50. Several factors were optimized during the development of binding protocols for each chip, including chip pretreatment, sample binding and wash. The data from

this study suggest that the detection and further characterization of urinary protein levels using SELDI-TOF mass spectrometry can provide specific proteomic-based biomarkers of exposure for acrylamide. (*Disclaimer: Mention of company names and/or products does not constitute endorsement by the Centers for Disease Control and Prevention (CDC). The findings and conclusions in this abstract have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent any agency determination or policy.*)

Proteomics-based Biomarkers of Acrylamide and Glycidamide Exposure Using *In Vitro* Blood Adduct Formation and SELDI-TOF Mass Spectrometry

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Acrylamide (CAS 79-06-1), a widely used industrial chemical, which is also found in thermally processed food, is known to produce neurotoxicity and reproductive effects. Acrylamide has been classified as a probable human carcinogen. The development of novel proteomic-based biomarkers to evaluate dermal exposure would be of value in assessing potential toxicity in workers. Bioactivation of acrylamide is reported to occur by CYP2E1 oxidation and GSH conjugation. Protein and DNA adducts have been reported for both acrylamide and glycidamide (5694-00-8), its oxidative metabolite. In the current study the reaction products of acrylamide and glycidamide with human ∀- and ∃-globins or albumin, obtained through *in vitro* incubation, were studied using Surface Enhanced Laser Desorption Ionization Time of Flight (SELDI-TOF) mass spectrometry. The study showed that the reactivity of glycidamide with albumin, ∀-globin and ∃-globin was much more than measured after incubation with acrylamide. After 12 hours the glycidamide-adduct levels for albumin > ∀-globin > ∃-globin, but significant adduct formation for acrylamide was measured only for albumin. The adduct levels continued to increase during the 72-hour test period. The data from this study suggest that multiple reactive sites exist for these proteins and that further characterization using SELDI-TOF mass spectrometry measurement of protein adducts in tryptic digests can provide specific proteomic-based biomarkers of exposure for acrylamide or glycidamide. (*Disclaimer: Mention of company names and/or products does not constitute endorsement by the Centers for Disease Control and Prevention (CDC). The findings and conclusions in this abstract have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent any agency determination or policy.*)

Development and Evaluation of an HPLC Method for the Simultaneous Quantification of Urinary Acrylamide and Its Primary Metabolite, N-Acetyl-S-(2-carbamoylethyl)cysteine

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A procedure for the quantification of urinary acrylamide and its primary metabolite, N-acetyl-S-(2-carbamoylethyl)cysteine (NACEC), using high performance liquid chromatography (HPLC) was evaluated. Acrylamide is found in the industrial work environment as well as being a component in many popular processed foods. The toxicity of acrylamide is of concern and the use of its primary metabolite as a biomarker of dermal exposure would be of value in the prevention of occupational disease and risk assessment. Urine collection and testing would offer a noninvasive means of accessing exposure. In this work, an evaluation of extraction

conditions using solid-phase extraction (SPE) of the two target analytes from spiked urine was conducted. Recovery was optimized for this procedure. Quantification of the two analytes was achieved using HPLC with a reversed-phase column (Phenomenex Synergi Hydro-RP) and gradient elution with a water/acetonitrile mobile phase containing 0.05% (v/v) ammonium formate, pH 2.6. Mass spectrometric detection using single ion monitoring (SIM) at m/z 72 for acrylamide and m/z 233 for the NACEC metabolite. Demonstrated accuracy for this procedure's recovery study was good; mean recovery varied between 97 to 108% for acrylamide and 97 to 102% for NACEC for various levels in urine. Precision as measured by relative standard deviation (RSD) during the recovery studies was 10% or less using fortified urine samples. The limit of detection (LOD) for this test procedure was calculated to be 0.03 µg/mL for acrylamide and 0.2 µg/mL for NACEC in urine. This method offers the ability to determine urinary levels of acrylamide and NACEC simultaneously, which has not been reported thus far in the literature. (*Disclaimers: Mention of company names and/or products does not constitute endorsement by the Centers for Disease Control and Prevention (CDC). The findings and conclusions in this abstract have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent any agency determination or policy.*)

Comparison of Human Genomic DNA Isolated from Blood, Urine, Buccal Cells and Saliva: Yield and Successful Performance in PCR, RFLP and Real Time PCR Genotyping Assays

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This study investigated the yield of DNA obtained from blood, urine, buccal cells and salvia including the genotyping performance of the DNA in polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP) and Real Time PCR (TaqMan™) methodology. Traditionally, human whole blood is used as the source of large quantities of high quality DNA. However, blood collection is an invasive technique and is not always the most convenient method to use in support of risk assessment studies. Five volunteers donated the selected biological specimens four times over a two month period. Genes important in detoxification of environmental and occupational toxicants were characterized. DNA was extracted from blood (0.3 ml), urine (10 ml), and buccal cells (10 ml mouthwash and two cheek swabs) using the Puregene DNA Purification Kit (Gentra Systems, Inc.), and saliva (0.2 ml) using the Oragene Collection Vial (DNA Genoteck, Inc.). The yield of DNA was determined using spectrophotometric methods. DNA yield showed wide inter-individual and intra-individual variability. The lowest DNA yield for each was: blood 2.2 µg, urine 0.4 µg, mouthwash 8.7 µg, cheek swab 0.3 µg, and saliva 2.0 µg. PCR assays were used to genotype *GSTM1* (wild type and *0/*0) and *GSTT1* (wild type and *0/*0). PCR/RFLP assays were used to genotype *GSTP1* I105V and A114V. In addition, the TaqMan™ assay was used to genotype *GSTP1* I105V. Ten to 200 assays could be conducted using the DNA extracts in PCR or PCR/RFLP. The number of TaqMan™ assays (2 ng DNA/assay) was dependent upon the quantity of DNA. All DNA extracted from blood, buccal cells and saliva produced excellent results. Genetic variants were concordant for all specimen types and genotyping methods for each individual. However, genotyping results for *GSTP1* I105V were obtained from only two of the DNA specimens extracted from urine and only in the TaqMan™ assay. Mouthwash and cheek swab cells are cost effective and offer a noninvasive specimen collection procedure for obtaining a reliable source of DNA to use in risk assessment studies. Saliva has more cost and difficulty involved in sampling and blood collection is invasive. This study demonstrated that there are alternative

procedures to obtain biological specimens to use as reliable sources of DNA for genotyping, instead of traditional blood sampling. Selection of specimen type is limited only by the number of genetic polymorphisms to be characterized and the ease and cost of obtaining the specimens. (*Disclaimers: Mention of company names and/or products does not constitute endorsement by the Centers for Disease Control and Prevention (CDC). The findings and conclusions in this abstract have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent any agency determination or policy.*)

In Vitro **Toxicity Assessment of Silver Nanoparticles in Rat Alveolar Macrophages**

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This study was conducted to assess the toxicity of nanosized silver particles (Ag-15 nm, 30 nm and 55 nm) in rat alveolar macrophages. For toxicity evaluations, cellular morphology, mitochondrial function (MTT assay), cell viability (LDH assay) and reactive oxygen species generation (ROS) were assessed after 24-hours under control and exposed conditions. The morphological appearance of control and exposed cells were observed by phase contrast microscope and the uptake of nanoparticles was also observed using the CytoViva Ultra Resolution Imaging (URI) system. The morphology of cells exposed to silver nanoparticles displayed abnormal size and irregular shape. It was also noted that agglomerates of nanoparticles were surrounded by macrophages; some attached to the cell membrane. Further analyses of Cytoviva images exhibit the uptake of agglomerates into macrophages. The results of the biochemical studies revealed that cells exposed to Ag-15 nm and 30 nm for 24 hours showed a dose-dependent decrease in mitochondrial function. Calculated EC_{50} values from MTT data indicate that Ag-15 nm and Ag-30 nm are more toxic at lower concentrations when compared to Ag-55 nm. In correlation with decreased mitochondrial function and subsequent lack of ATP production, cell viability assays also demonstrated a sizeable decrease in the number of viable cells exposed to Ag-15 nm and 30 nm, including the lowest dose (5 µg/ml). ROS date further supported a size- and dose-dependent relationship, with a 15.16$\pm$ 5.77 fold increase in ROS generation at 50 µg/ml of Ag15 nm. Preliminary apoptosis results reveal a size-dependent increase in caspase 3&7 enzyme activity; an indicator of apoptosis. In conclusion, MTT, LDH and ROS data, reveal particle size is a potential factor in assessing toxicity and Ag-15 nm is found to be the most toxic when compared to larger nanoparticles (30 nm and 55 nm). The study also suggests that the mechanism of toxicity is likely due to the generation of reactive oxygen species, inducing oxidative stress and resulting in apoptosis.

Nickel Absorption Following Water Ingestion in Adults: A Probabilistic Approach to Estimation of Bioavailability

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Estimation of health risks associated with exposures to nickel in drinking water requires accurate estimates of nickel bioavailability. Bioavailability of water soluble nickel is higher when exposure occurs in the fasted state than when exposure occurs with meals, and decreases as the time between exposure and the meal decreases. Therefore, average bioavailability of water-borne nickel would be expected to be influenced by variability in the daily temporal patterns of drinking water and meal ingestion. In the current study, we used Monte Carlo

simulation and meal consumption data from NHANES III, along with experimentally-derived estimates of changes in bioavailability of nickel in subjects who consumed nickel at various times before or after meals, to derive estimates of a meal-weighted daily average bioavailability of water soluble nickel in the non-institutionalized U.S. adult male population. The model predicted meal-weighted absolute bioavailability estimates ranged from a low of 3.4%, for people who ingest 100% of their total daily drinking water intake at meal times, to as much as 17% for people who ingest all of their water between meals. A sensitivity analysis of the meal-weighting nickel bioavailability model indicated that the estimate of the bioavailability of nickel was most sensitive to the parameter that represented the percent of exposure that occurred at meal times and the number of meals eaten per day. The model was not sensitive to the number of exposure events per day, or the intra-individual correlation in absorption fraction.

A Carcinogen Bioassay for Complex Mixtures of Polycyclic Aromatic Hydrocarbons

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Complex mixtures of combustion products contain many carcinogens, including polycyclic aromatic hydrocarbons. Assessing the carcinogenic potency of mixtures by a conventional mouse skin tumor carcinogenesis protocol (CC) is time-consuming and expensive. Initiation-promotion protocols are of uncertain accuracy in the prediction of cancer outcome. We evaluated a mouse skin model using a dominant-negative p53 mutant mouse (Vp53) in an initiation-progression protocol (IP), concurrently applying a mixture and a promoter, 12-*O*-tetra-decanoylphorbol-13-acetate (TPA), for 26 weeks. The mixtures were also tested with Vp53 wild type mice in a CC protocol. Three dose levels of Benzo[*a*]pyrene (BaP) were first tested to confirm the sensitivity of the models. Four mixtures were then evaluated: a National Institute of Standards and Technology SRM 1597 Coal Tar mix (CT), a 7*H*-dibenzo[*c,g*]carbazole (DBC) and BaP mix (M1), two dose levels of coal tar creosote (Cr), and a DBC, BaP and CT mix (M2). No tumors developed in control mice. The percentage of mice with tumors was more than 50% in all mixture treatment groups except M1 and M2 CC. The tumors in CT first appeared at 13 weeks in IP and 15 weeks in CC, with 18 and 25 week latent periods, respectively. The M1 IP tumors first appeared at 10 weeks, with a 20 week latent period; there were no tumors in CC. The first M2 tumors appeared at 12 weeks in IP and 17 weeks in CC, with latent periods of 15 and >26 weeks, respectively. 10 mg Cr tumors first appeared at 11 weeks in IP and 16 weeks in CC, with 17 and 24 week latent periods, respectively. The 20 mg Cr tumors first appeared at 8 weeks in IP and 12 weeks in CC; latent periods were 15 and 16 weeks, respectively. In comparison to conventional protocols the IP protocol results in shorter latent periods and time to first tumor for complex mixtures. *(U.S. EPA Star Grant)*

Scientific Rationale for Deriving Database Uncertainty Factors for Safe Dose Estimates that are Protective of Children

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Potential effects of toxicants on childhood, reproduction, and development are regularly explored when setting safe exposure levels [e.g., reference doses or concentrations (RfDs or RfCs)] that are expected to protect children's health. In setting such safe exposure levels, the

U.S. EPA commonly uses an uncertainty factor, routinely referred to as database uncertainty factor, UF_D, of 3 or 10 for varying degrees of database incompleteness when information suggests that developmental, reproductive or developmental neurotoxicity may be the critical effect in the absence of specific information. Since the current default value of UF_D is based on an analysis of data sets for a fair number of pesticides in the late 1980s, we compiled toxicity data from experimental animals for classes of chemicals with diverse mechanisms of action and used this compilation to explore the procedure for deriving safe doses when no adequate human or animal reproductive or developmental studies are available. The compilation provides opportunity to investigate whether the current defaults are supported by an updated comparison of study effect levels for systemic versus reproductive and developmental endpoints on other chemical classes and a larger number of chemicals. It also provides an estimate of how often the most sensitive endpoint would be missed in the absence of reproductive or developmental studies, and directly relates to the value of UF_D. Based on the present compilation, the default uncertainty factor of 10 is not always consistent with 95% coverage of the distribution of the chemicals used in the analysis when reproductive and developmental no-adverse-effect levels (NOAELs) are missing and a chronic experimental animal NOAEL is available in only one species. Although the U.S. EPA default UF_D may not be adequate for some combinations of studies, particularly for protecting against effects on reproduction, it appears more than adequate for other combinations of studies. *(Keywords: database uncertainty factor, children, reproduction)*

Evaluation of Skin Barrier Creams to JP-8 Irritation in New Zealand White Rabbits (*Oryctolagus cuniculus*)

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Skin Irritation, as well as skin and systemic toxicity due to accidental JP-8 jet fuel exposure remains a concern for the U.S. Air Force and their fuel handling personnel. Personal protection equipment and clothing (PPEC) is a necessity under normal operations but requirements to meet workload goals can lengthen individual exposure causing PPEC failure, especially during extended operations. To ensure work conditions result in the lowest JP-8 exposure, our goal was to add a skin-enhancement barrier cream to the PPEC armament. Initially we needed an over-the counter (OTC) product or cream claiming to have skin enhancement and/or barrier properties, which could be quickly fielded. To determine the best OTC creams, a number of creams were tested *in vitro* on both silastic membrane and dermatomed pig skin. The five best creams were selected for *in vivo* testing on New Zealand White Rabbits to determine the degree of protection against JP-8 irritation. The Barrier creams were applied to the skin of shaved rabbits then exposed to 0.5 ml of JP-8 for four hours at selected sites using a 2 cm Hilltop Chamber. The eleven sites included one negative control site (skin and hilltop chamber only), one control site (barrier cream and hilltop chamber only), three positive controls (JP-8 and hilltop chamber only) and three sample sites (JP-8, hilltop chamber and barrier cream applied). Also included on each rabbit were three sites of unexposed bare skin. Readings were taken both with a colorimeter and visually according to the Draize Irritation Scale. Histopathology samples were also taken at the exposure end point. These sites were evaluated at eleven time points over 72 hours. Three creams have shown protection against JP-8 irritation, while other creams that protected against JP-8 penetration *in vitro*, do not provide sufficient protection for JP-8 irritation on rabbit skin *in vivo*.

Use of BMC Modeling and Categorical Regression to Evaluate Human Acute Sensory Irritation from Chloropicrin Vapor

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Benchmark concentration (BMC) and categorical regression modeling were conducted for chloropicrin, with the primary purpose of investigating a 1-hour exposure limit. BMC modeling was conducted on data from Cain (2004), which evaluated early sensory-perception effects of chloropicrin in human volunteers. The BMC_{10} and $BMCL_{10}$ for ocular irritation, the most sensitive endpoint, were 110 and 73 ppb, respectively. Categorical regression of the broader data set was conducted to evaluate concentration-duration-response relationships. Results of categorical regression analysis indicated that the 1-hour EC_{10} for humans is 112 ppb, and the lower bound on this value is 90 ppb. The EC_{10} is remarkably consistent with the BMC_{10} for ocular irritation. The BMC models provided the best fits to the data, and thus the BMC results are recommended as the basis for the development of exposure limits. The results of the categorical regression modeling can be considered an upper bound on the response in the 8-24 hour range and should not be used for extrapolation to significantly longer time ranges. The actual exposure limits for the 8-24 hour range may be closer to, or identical with, the 1-hour value. Based on several alternative analyses that considered the impact of various decisions in the analysis, a reasonable range for the point of departure (POD) is 40-120 ppb. Because the $BMCL_{10}$ is a NOAEL surrogate derived from a human study, the only uncertainty factor (UF) to be considered is for human variability. Based on data on human variability in response to sensory irritants, a UF of 2 is our best judgment of the appropriate UF, although the actual value of a UF that is adequately protective for variability and sensitive populations may be 1 or 3. A UF of 2 results in a 1-hour exposure limit of 40-50 ppb that protects sensitive populations. It should be noted, however, that not all combinations of UF and POD are appropriate.

Key Steps Needed for Evaluating Sensitive Toxicological Effects for Hazard Identification

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Hazard identification is a key component of risk assessment. The most important steps in the hazard identification process are finding the critical effect of a chemical, known also as the most sensitive adverse effect, and identifying the known precursor event. In order to be applicable to hazard identification, both the critical effect and the known precursor event must be relevant to humans for exposure route and duration of concern. Because toxicity data may often be generated for purposes other than risk assessment, risk assessors must evaluate the relevance and suitability of the published studies to determine whether the data support risk assessment activities. This presentation will outline key steps to identify critical effects, including: (1) evaluating the experimental designs and results of human and animal studies, (2) examining the duration and route of exposure, (3) evaluating the biological and statistical significance of adverse effects, (4) extrapolating animal data to humans and (5) selecting the most sensitive effect relevant to humans. We will discuss application of available published guidelines to evaluate consistency of data across species, mode of action, and dose-response for selection of

critical effects in principal and supporting studies. (*This presentation does not necessarily reflect the views and policies of the U.S. EPA.*)

Evaluating Data for Determining an Oral Reference Dose (RfD) and Reference Concentration (RfC) for Bromobenzene

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Bromobenzene appears on the U.S. EPA Office of Water Contaminant Candidate List (CCL), but is absent from the Integrated Risk Information System (IRIS). U.S. EPA is in the process of developing a Toxicological Review for bromobenzene that will include the justification for hazard identification and dose response assessment. This Toxicological Review will incorporate the existing health effects and risk information data for bromobenzene. No data were located regarding the toxicity of bromobenzene in humans. Animal studies identify the liver as the most sensitive target of oral and inhalation exposure to bromobenzene. Numerous mechanistic studies in animals demonstrate that hepatotoxicity is associated with the metabolism of the parent compound and cytotoxicity may result from modifications of hepatocellular macromolecules by one or more reactive metabolites. Nephrotoxicity has also been observed in animals following acute exposure at higher doses than the lowest hepatotoxic dose. Using U.S. EPA Benchmark Dose Software (version 1.3.2), data for several hepatic endpoints in mice and rats were modeled. This poster will evaluate the data that are available in male and female mice and rats following oral and inhalation exposure for a determination of an RfD and RfC. This approach will provide a better understanding of the toxicity of bromobenzene and will aid in making informed risk-based decisions for protection of human health. (*This presentation does not necessarily reflect the views and policies of the U.S. EPA.*)

Detection of Nanosize Particles in Living Cells Using an Advanced Illuminating System Microscope

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Nanotechnology involves the creation and manipulation of materials at the nanoscale level to create unique products that exploit novel properties. Recently, nanomaterials such as nanotubes, nanowires, fullerene derivatives (buckyballs) and quantum dots have received enormous national attention in order to create new types of analytical tools for biotechnology and the life sciences. In general, nanomaterials are of interest to the Air Force because of their potential use in electronics, sensors, munitions and energetic/reactive systems involved with the advancement of propulsion technology. However, a detailed understanding of their interaction with living cells is needed in order to identify their biohazards. The main objective of the study was to detect nanoparticles in living cells. In the present study, the CytoViva150 Ultra Resolution Imaging (URI) system with advanced optical illumination system was applied to follow the uptake, translocation and distribution of various nanosized particles in various living cells under physiological conditions. The experimental results demonstrated that silver 25 nm, Al 30 nm, Mn 40 nm nanoparticles were effectively internalized by liver cells, macrophages and PC-12 cells respectively. The distribution of these particles individually and in aggregation was found in the cells. Additionally, bright aggregates were also found attached to the membrane

outside of the cells. It appears that aggregation is an intrinsic property of these particles and significantly reduces the number of individual particles in the cells. However, problems of excessive agglomeration in the nanoparticles were partially reduced by treating the particles with an anionic surfactant (.1% SDS) and sonicating the particles for several seconds before exposure. This resulted in less conglomeration and more finely separated particles thereby increasing the amount of particles that were internalized by the cells. Further studies are under investigation to characterize dynamic properties of these particles such as size, dimension and particle distribution in the cells.

Analytical Chemistry of Metalworking Fluids for Dermal and Inhalation Toxicology

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The National Toxicology Program (NTP) is currently evaluating metalworking fluids (MWF), and NIOSH has been provided MWF samples used in the NTP study. In addition, NIOSH researchers are also studying MWF toxicology by local lymph node assays (LLNA). An analytical chemistry method for MWFs was needed for these studies and the ongoing method development was described in this poster with some preliminary data. The metal working fluids were analyzed by high performance liquid chromatography with evaporative light scattering and ultraviolet light detection (HPLC-ELSD-UV), preparative HPLC with fraction collection, and gas chromatography with mass spectrometry (GC-MS) using three modes of ionization - electron impact (EI), positive chemical ionization (PCI), and negative chemical ionization (NCI). The analytical methods provided chemical composition information on commercial metalworking fluids not available in the manufacturers' material safety data sheet (MSDS). MWF components were identified by molecular formula, molecular structure, and CAS number. The separation and qualitative identification of the components in one new MWF concentrate is presented.

Using analytical HPLC-ELSD-UV, the MWF components separated according to polarity, bases first and oils last. Their relative mass was measured using evaporative light scattering detection (ELSD). Some components were also detected using UV absorption. UV-ELSD active internal standards provided retention indices for unknown peaks. An overlay of 9 HPLC-ELSD chromatograms exemplified its use in the screening and classification MWFs. This method was also found to be applicable to MWF concentrates diluted with water as prepared for use. Using GC-Mass Spectrometry, the MWF components were separated by molecular weight and the molecular structures were identified using EI mass fragmentation patterns matched against an NIST library. Greater than 95% spectral match considered positive. Negative and positive chemical ionization mass spectra provided molecular weight data. Using preparative HPLC, MWF samples were concentrated, separated into components, and the components isolated in solution fractions. Further analysis was done on the fractions, like GC-atomic emission detection (AED) to confirm the identity of chlorine in chloroparaffins. Additional atomic and molecular spectroscopy was used as needed to confirm and/or identify unknowns.

Using Human Life Stage PBPK/PD Model Predictions of Perchlorate-Induced Iodide Inhibition to Inform Risk Assessment in Sensitive Populations

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The U.S. Environmental Protection Agency (U.S. EPA) established a perchlorate reference dose (RfD) of 0.0007 mg/kg-day and placed this value into their Integrated Risk Information System (IRIS). The National Research Council report "Health Implications of Perchlorate Ingestion", released in January of 2005, supports this value as protective for all populations. Iodide inhibition was considered to be the key biochemical event preceding disruption of thyroid hormone homeostasis. The RfD was based on the No Observable Effect Level (NOEL) of 0.007 mg/kg-day, which resulted in no significant iodide inhibition in normal adults. An uncertainty factor (UF) of 10 was applied to the NOEL to account for intraspecies variability, including life-stage specific susceptibility. Recently, an existing suite of physiologically based pharmacokinetic/ pharmacodynamic (PBPK/PD) models across life-stages in the rat and in the adult human was expanded to describe inhibition kinetics during perinatal development in the human. Chemical-specific parameters were estimated from life-stage and species-specific relationships established in the previously published PBPK/PD models. The human perinatal models successfully simulate literature radioiodide data for gestation and lactation, as well as perchlorate data from populations exposed to contaminated drinking water. These validated models were used to examine the effect of developmental stage on the susceptibility to thyroid perturbation across a range of doses. At environmentally relevant doses, the perinatal woman, fetus and nursing infant are predicted to have higher blood perchlorate concentrations and greater thyroid iodide uptake inhibition than either the non-pregnant adult or older child. Based on predicted iodide inhibition, the most susceptible life-stage was determined to be the fetus. At exposure levels equal to the NOEL and RfD, the PBPK/PD model predicted inhibition in fetuses is within normal variation (less than 10%) and insignificant (less than 1%), respectively, indicating that the RfD is in fact protective of the populations most sensitive to thyroid inhibition.

Decision Support Framework for Assessing Occupational Health Risk in Indoor Environments

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Characterization of the health and environmental concerns associated with potentially hazardous materials released into the air during industrial operations in indoor environments is important for many industrial and military operations. Traditional fate and transport models may not be appropriate since exposure occurs near an emission source, resulting in complex gradients and flow patterns. Moreover, toxicology of many emerging materials (such as nanoparticles, beryllium, etc.) is not well understood. Nevertheless, protection of workers and service members and conducting operations in a manner that minimizing risks and takes into consideration criticality of the military mission is an important current task. This presentation reports a conceptual model of a Health and Environmental Simulation (HES) environment developed to assist the Air Force in evaluating potential health risks to personnel engaged in the servicing and maintenance of aircraft. Computational fluid dynamics (CFD) modeling was demonstrated to be a capable and powerful tool for estimating the dispersion of contaminant emissions generated by typical industrial operations such as sanding, surface coating, and

liquid tank operations, both within the immediate area of the release (~2 ft) and at other locations in the near vicinity where others may work (prompting concerns over incidental exposure). Preliminary software modules were designed to surround the key CFD element to create model input files from user specifications and process output data in a manner consistent with occupational safety data. In conjunction with traditional hygiene surveys, HES modeling can be useful in determining the level of protection needed to protect various workers, and hence to assist in the development of standard operating procedures for repairs and servicing. Embedding HES within a Multi-Criteria Decision Analysis framework allows managers to evaluate trade-offs between potential risks to workers, costs, project deadlines and other factors relevant to dynamic resource allocation in the manufacturing environment. We anticipate MCDA/HES applications can benefit many complex, poorly understood contaminant exposure problems involving traditional industrial processes, the health care industry (biocontaminants), and emerging nanotechnologies.

Use of Multi-Criteria Decision Analysis Tools to Facilitate Assessment of Exposure to Materials of Evolving Regulatory Interest (MERIT) and Low-Level Chemical Agents

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Elucidation of low-level chemical warfare agent exposure, as well as exposure to materials of evolving regulatory interest (MERIT), requires multiple sets of information due to both the variability in exposure and the limited database of relevant experimental toxicity studies. The U.S. EPA and other agencies generally use a weight-of-evidence approach in evaluating the potential carcinogenicity and toxicity of chemical agents. Traditionally, assessors weigh various lines of evidence and apply professional judgment and/or calculations to decide where the weight of evidence lies – that is, whether the various lines of evidence point to potential risk in the case of each receptor or not. Even though weight-of-evidence considerations may use some quantification, this approach often results in arbitrary weight selection (e.g., conservative bias) and thus in risk estimates that include an unquantified degree of uncertainty and potential bias. We argue that weight-of-evidence approaches may be useful for assessing exposure and risks to low-level chemical warfare agents, but a limited knowledge base and high uncertainty and variability in their basic properties requires coupling traditional weight-of-evidence assessments with multi-criteria decision analysis (MCDA) to support toxicity assessment and regulatory decision making. MCDA offers a rigorous and consistent approach. While MCDA is often used to incorporate social factors into decision-making processes, this presentation will illustrate the potential of MCDA to facilitate purely technical evaluation of the multiple lines of evidence used in typical human health risk assessment for low-level chemical warfare agents.

Pharmacokinetic Characterization of Potentially Susceptible Subpopulations: Physiologically Based Pharmacokinetic Modeling in Children

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As a result of enhanced exposure of internal targets and their developmental immaturity, young children may represent the subpopulation particularly susceptible to the effects of some environmental chemicals. Their absorption, distribution, metabolism, and excretion (ADME) can be fully described and/or predicted by physiologically based pharmacokinetic (PBPK) models.

The modeling approach of several successful PBPK models in children has been reviewed and analyzed. The goal of this presentation is to review the resources and to compile from the available literature a set of PBPK input parameters that characterize the unique ADME of chemicals in children and could be applied in PBPK modeling. The modeling methodology presented here may be used in life-stage-specific derivation of toxicity values and in chemical risk assessments for children exposed to environmental chemicals.